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METHYLTHIOURACIL IN THE TREATMENT OF THYROTOXICOSIS

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The purpose of this paper is to summarize the results achieved in using 4-methyl-2-thiouracil in the treatment of 61 cases of thyrotoxicosis and to report the results of maintenance therapy with the same drug.

Since Astwood[1] in 1943 published the first reports on the use of thiourea in thyrotoxicosis many attempts have been made to synthesize a potent and at the same time a non-toxic drug. The ideal preparation is yet to be found. Methylthiouracil is a Danish contribution to the search for such a drug and was suggested by Prof. K. A. Jensen of the University of Copenhagen in 1944, and has been used clinically in Denmark, on a rather large scale, ever since. Coincidentally, Chapman,[8] and O'Donovan,[11] in England, were trying the same compound but without knowledge of the Danish experiments because of war conditions. There are only a few reports from England upon its clinical value. In Sweden and Norway methylthiouracil has been used to some extent.

The experimental work has been done by Freisleben,[14] and Thyssen,[38,39] in Denmark, and Chapman,[8] in England. It was shown that methylthiouracil had a stronger goitrogenic effect on rats than thiouracil, whereas the toxic effect (lethal dose for mice) was the same. Propylthiouracil had 10 times as great a goitrogenic effect as thiouracil[2] but this does not hold true of its antithyroid action in man. The reason for this was recently shown by McGinty[25] who proved that methylthiouracil is not conjugated in human blood while the reverse is true of propylthiouracil, resulting in its partial inactivation. Christensen[9] has also shown that several of the thiouracil derivatives are bound to the plasma proteins in blood. Thirty-five % of methylthiouracil can be recovered by ultrafiltration and only 5% of the propylthiouracil can be so recovered. A practical proof of this is the fact that at present the small doses of propylthiouracil proposed by Astwood,[2] based on the marked goitrogenic effect, are being increased while the methylthiouracil dosage is rapidly diminishing and is nevertheless causing a good antithyroid effect. The doses of these 2 drugs are now nearly equal. The reason, however, methylthiouracil received preference in Denmark was not the greater potency of methylthiouracil but the fact that its manufacture was easier and less expensive.

Material. Table 1 gives an impression of the different types of cases. It will be noticed that the ratio of women to men is nearly 4 to 1, which is a little more than that usually seen in Denmark.[36] Of the patients belonging to the old age group,

TABLE 1.—GROUPING AND CLASSIFICATION OF THE ENTIRE MATERIAL

Age group (years):	18 to 40						41 to 50						51 to 72					
Mean age for group (years):	31						45						59					
Total cases:	61						14						18					
Women:	51						11						14					
Men:	10						3						4					
Type of goiter:	Diffuse			Nodular			Diffuse			Nodular			Diffuse			Nodular		
Women, diffuse goiter . . 40	24						8						8					
Men, diffuse goiter . . 9	3						3						3					
Women, nodular goiter . . 11				2						3						6		
Men, nodular goiter . . 1																1		
Severity of disease:	+	++	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++
Women, + ... 28	13			1			5			3			5			1		
Women, ++ 17		7			1			3						1				
Women, +++ 6			4												5			
Men, + ... 3							2						1					
Men, ++ 4		2						1						1				
Men, +++ 3			1															1
In all:																		
Diffuse (+26, ++15, +++8) . 13	9	5					7	4					6	2	3			
Nodular (+5, ++6, +++1) . .				1	1					3						1	5	1

+ = BMR, +15 to +49% and 2 main symptoms (see text). ++ = BMR, 50% and 3 main symptoms. +++ = BMR, +50% and 5 main symptoms.

a surprisingly large proportion are men, nearly one-third. As would be expected, diffuse goiter is by far the most common form. The incidence of nodular goiter increases with increasing age. By nodular goiter is understood a goiter with an irregular surface or a single nodule, no matter whether it is cystic or of a more elastic character. Most of the goiters were small and barely palpable. Secondary thyrotoxicosis occurred in 3 patients who had a diffuse goiter and in 5 patients who had a nodular goiter. Three of the patients were treated for relapses; 1 case having a diffuse goiter and 2 having nodular goiter.

The degree of severity is graded in the following way: +++ means that the patient had all the 5 main symptoms: exophthalmus, goiter, tremor, tachycardia, motor and/or psychic instability, and a basal metabolism rate of more than +50. The ++ group had only 3 of the main symptoms (exophthalmus lacking for the most part) and a basal metabolism rate of more than +50. The + group had only 2 main symptoms and a basal metabolism rate between +15 and +50. This is a somewhat arbitrary manner of dividing the material, as patients belonging to the + group occasionally were just as ill as those in the +++ group. It has, however, the advantage of not being dependent on subjective impression. In questionable cases a response to iodine determined whether the patient had a true thyrotoxicosis or not. Apart from this the patients showed a variety of other thyrotoxic symptoms such as: diarrhea, weakness, weight loss, thirst, etc. One-half of the cases belong to the + group; the +++ group being the smallest, 9 out of 61. There are comparatively more men in this group and the severe cases are only found in the young and in the old age group; the ++ group being evenly distributed among all 3 age groups. Half of the nodular goiters belong to the ++ group, and only 1 nodular goiter belongs to the +++ group.

Technique. Since 1944 we have used methylthiouracil at the County Hospital in Odense, but the indications for its use have been somewhat varied. Because of reports on the sensitivity to the drug we at first decided to use methylthiouracil solely for treatment of iodine-resistant cases and, as soon as a good response was obtained, to submit the patients to subtotal thyroidectomy. Later we treated all our cases of thyrotoxicosis with methylthiouracil with the purpose of continuing a maintenance dose if there were no contraindications, such as: (1) intrathoracic goiter, (2) a very large and increasing goiter with pressure symptoms, (3) failure by patients to submit to regular basal metabolism rate control.

The following routine was used: All patients were hospitalized and basal metabolism rate determinations were done twice a week. The pulse rate was recorded daily. The patients had no special diet, some received a few sedatives. The leukocyte and differential counts and a test for urobilin in a 1:10 dilution of the urine were done twice a week. The patients stayed in the hospital, with a few exceptions, until the initial dose of methylthiouracil could be lowered, following a fall in basal metabolism rate, a weight gain, and subjective improvement. Thereafter the patients were followed at 2 to 3 week intervals; later when the basal metabolism rate approached a normal level, the patients were followed at 1 to 2 month intervals. After treatment was discontinued, basal metabolism rate was done every 2 to 3 months; leukocyte and differential counts and tests for urobilin were done at the same time and the patients were urged to report immediately any rash, fever, malaise or sore throat. With few exceptions the patients have willingly followed this routine and have been glad to avoid surgical procedures.

Results. The results can be very much clarified if one divides the whole material into 2 separate groups as shown in Table 2. The first group contains all those patients who previously had experienced a long period of iodine treatment and had become more or less iodine-resistant. As already mentioned, these patients were the first to be treated and, as it was recommended at that time, received a rather large dose (1 gm. of methylthiouracil a day); and later, if they were still resistant to treatment, a still larger dose of the drug was used. It is impossible from the graphs to decide whether the larger doses did help, but it does not seem that they did so in the case which is described later on. (The case with the severe neurologic symptoms described under toxic effects.) From what we now know one would expect some decline in the basal metabolism rate from long-continued treatment with methylthiouracil irrespective of the dosage. It is also evident from the animal experiments that when a certain dosage of methylthiouracil is exceeded the goitrogenic effect does not increase.[39] The same clinical impression is reached by Magnusson.[24a] The largest average doses which were given in the initial period of treatment were 1.2 gm. a day (1 patient had 0.4 gm.; 2 patients had 1 gm.; 2 patients had 1.4 gm.; 1 patient had 2 gm.; 1 patient had 2.2 gm.). Because of these dosages which we now would consider as huge, reactions were very frequent and in some cases even severe, though we did not observe any cases of agranulocytosis in this group. Several authors[19,24,42] have stressed the increase in dosage to be the cause of increased sensitivity. There is no doubt, that more of the patients could have been carried through on methylthiouracil treatment if the patients' tolerance to a smaller dose had been tried. After the first signs

TABLE 2.—RESULT OF METHYLTHIOURACIL TREATMENT WITH SPECIAL ATTENTION TO PREVIOUS IODINE TREATMENT

	No. cases	A Treatment effective	B Treatment partly ineffective	C Treatment discontinued (toxic reaction)	Average daily dose in the initial period (gm.)
Iodine for a long period, previous to treatment with methylthiouracil	18	7 (39%)	3	8 (44%)	1.20
No previous iodine	43	37 (86%)	1	5 (12%)	0.57
Totals	61	44	4	13	

of intoxication had occurred. Table 5 shows that we only did this test in 8 out of 14 cases. We now do it routinely.

The second group contains all those patients who did not receive iodine to any extent before methylthiouracil treatment was commenced, although 3 patients had iodine for a week for diagnostic purposes and responded well. This group is the largest group and received gradually decreasing daily doses during the initial period. The first patients treated received doses as follows: 1 patient 1 gm., 2 patients 0.8 gm., the following 26 patients 0.6 gm. and 5 patients 0.4 gm. The last 3 patients have been treated with 0.2 gm. (2 cases) and 0.1 gm. (1 case), and it is our intention in the future not to exceed 0.4 gm. for the first 4 weeks, even if the case has previously been treated with iodine. The average initial daily dose has been 0.57 gm. in this group. The therapeutic results in this group are comparable with other reports on methylthiouracil and give a true picture of what can be expected from this form of treatment. The above-mentioned reduction in dosage has worked satisfactorily and the same experience has been encountered by McGullagh.[26] This reduction in dose will perhaps result in a smaller number of reactions, though it is uncertain if it will reduce the danger of agranulocytosis. The comparison of the results shown in Table 2 and the literature on this subject will be commented upon below.

Nearly all authors seem to agree that prolonged iodine treatment prior to methylthiouracil treatment causes a considerable prolongation of the time required for remission. This has been mentioned by several authors.[1,5,16,24,28,34,44] Others[10] have reported contradictory experiences and have demonstrated that thiourea, combined with iodine or even following previous iodine treatment, does not prolong the treatment; on the contrary, these drugs act as synergists. The possible explanation may be that our cases were iodine-resistant and not ordinary cases of thyrotoxicosis receiving iodine with good effect prior to methylthiouracil treatment. Figure 1 shows 14 cases who were iodine-resistant and who were treated with methylthiouracil following prolonged iodine treatment and who also for some time had iodine and methylthiouracil together. For comparison, the dotted lines represent 14 other cases chosen at random which were not treated with iodine and which had a

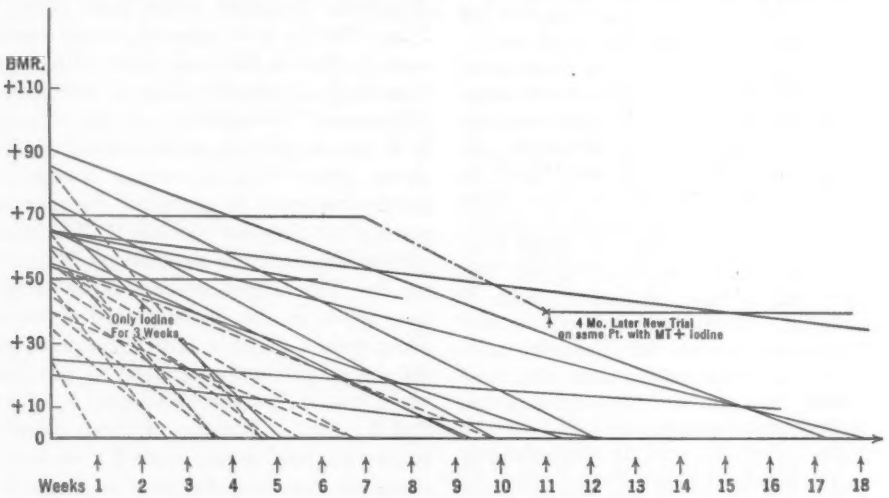
BMR.
+110
+90
+70
+50
+30
+10
0
Only Iodine For 3 Weeks
4 Mo. Later New Trial on same Pt. with MT + Iodine
Weeks 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

FIG. 1.—14 patients: iodine-resistant cases—other 14 patients: chosen at random, without previous iodine treatment.

similar initial basal metabolism rate. The different rate of response is quite evident from the curves and proves that iodine-resistant cases behave in a special way towards methylthiouracil treatment.

SUSTAINED REMISSIONS AND MAINTENANCE THERAPY. In maintenance therapy our principle has been the following: A gradual decrease in the dosage, not following any set scheme, and then, when the patients have done well for a month or so, on 0.05 gm. a day, withdrawal of the drug usually after an average period of treatment of 5 months.

TABLE 3.—COURSE OF CASES RESPONDING TO TREATMENT WITH METHYLTHIOURACIL
(Corresponds to Group A in Table 2)

					22 patients at present on methylthiouracil therapy	
		Sustained remission after withdrawal of drug	Preoperative treatment	Therapy discontinued (non-co-operative patients)	Reduction in drug dose successful but no attempt to withdraw drug	Sustained remission unsuccessful after reduction or withdrawal of drug
Number of patients: 44		16	4	2	10	12
		Cured 36.4%	Final evaluation impossible 36.4%			Relapses 27.2%
Duration of treatment (months)	Mean	5	3	4	6	12
	Max.	10½	4	6	14	17
	Min.	2	½	2	2½	3
Average length of follow-up after withdrawal of drug (months)	Mean	7½				
	Max.	2 in 12				
	Min.	3 in 2½				

Table 3 shows the results of methylthiouracil treatment up to date in 44 patients (Group A) from Table 2, in whom therapy was possible. These cases were divided into 3 main sub-groups:

1. Those who remained in remission even after removal of the drug for an average time of 7½ months, designated in Table 4 as "cured," in the sense that their thyrotoxicosis disappeared during the period in which the symptoms of disease were controlled by methylthiouracil. The criteria for the determination of a remission in this group have included: subjective relief, weight gain, disappearance of the elevated basal metabolism rate and decreased pulse rate. The rather scanty literature on this subject[4,29,35] has demonstrated that the relapses most commonly occur within 2 to 3 months after withdrawal of therapy, but that they may occasionally occur at the end of 6 months or even later. In our cases in sub-group 3, the mean time for relapses to occur, following cessation of treatment, was 2 months, the maximum time 6 months and the minimum time 1 month.

2. The next sub-group contains all the cases in which an evaluation of maintenance therapy is impossible, either (*a*) because of preoperative treatment or (*b*) because of therapy being discontinued as the patient was non-coöperative, or (*c*) because the treatment is still going on and it is impossible to decide upon the final results. This group is designated, "final evaluation impossible."

3. The third sub-group includes all patients who have had relapses after at least 3 months of treatment as the result of withdrawal of the drug; the average total length of treatment being 12 months. The average relapse occurred within 2 months after withdrawal of the drug. Some of these patients may later have a sustained remission, although some have failed twice on withdrawal of the drug. Those patients with 1 year of maintenance therapy not resulting in permanent remission ought to be operated upon. The average dosage of methylthiouracil given to these patients to keep them in remission was a little more than 0.1 gm. a day with a spread from 0.3 to 0.05 gm. This group is designated "relapses."

In Table 4 we have tried to relate the "cured" group with the type of goiter, severity, age, sex, complications, and the duration of the disease, with the thought in mind that thereby we might be able to

TABLE 4.—RELATION BETWEEN COURSE WHEN RESPONDING TO TREATMENT WITH METHYLTHIOURACIL AND THE TYPE OF GOITER, SEVERITY, SEX, AGE GROUP AND COMPLICATIONS AND DURATION OF DISEASE

									22 patients, at present on maintenance dose			
				Sustained remission after withdrawal of drug			Preoperative treatment	Therapy discontinued in non-coöperative patients	Reduction in drug successful but no attempt to withdraw	Unsuccessful sustained remission after reduction or withdrawal of drug		
The number and percentage in:			Each group:	16			4	2	10	12		
			This group:	Cured 36.4%			Final evaluation impossible		36.4%	Relapses 27.2%		
			Entire material:	Cured 26.2%						Relapses 19.7%		
		Incidence in:			Percentage in						Percentage in	
		This table	Entire material	Total	This table	Entire material				Total	This table	Entire material
Goiter:	Small to moderate	28	36	12	43	33	2	1	8	5	18	14
	Large diffuse	8	13	2	25	15	2	..	1	3	38	23
	Nodular	8	12	2	25	17	..	1	1	4	50	33
Severity:	+	26	31	10	38	32	..	2	7	7	27	23
	++	13	21	4	22	19	2	..	3	4	31	19
	+++	5	9	2	40	22	2	..	..	1	20	11
Sex:	Female	38	51	13	35	25	3	2	9	11	29	22
	Male	6	10	3	50	30	1	..	1	1	17	10
Age groups:	18 to 40	22	29	9	41	31	3	..	5	5	23	17
	41 to 50	10	19	5	50	26	..	..	2	3	30	16
	51 to 72	12	18	2	17	11	1	2	3	4	33	22
Complications*		24	38	6	25	16	3	2	7	6	25	16
Duration of disease prior to treatment with drug			Mean	¾ year			1½ years	..	3½ years	2 years		
			Max.	1 for 24 months			1 for 49 months	..	1 for 288 months	1 for 62 months		
			Min.	1 for 2 months			1 for 2 months	..	1 for 2 months	1 for 3 months		

+ = 2 cardinal signs and BMR, +15 to +49%. ++ = 3 cardinal signs and BMR, +50%. +++ = 5 cardinal signs and BMR, +50%.
*Complications: cardiovascular or severe chronic disease such as diabetes.

predict which cases would fall into the "cured" group, and which would belong to the "no lasting cure" group. First of all, it may be pointed out that in starting methylthiouracil treatment we can only expect one-fourth to fall into the "cured" group. This figure is no doubt too small as the use of smaller doses of methylthiouracil will probably reduce reactions and add to the "cured" group, even if some few patients may later relapse.

diseases from which the patients suffered during the time of treatment, whether connected with the thyroid status or not. There were just as many patients who suffered from complications in the "cured" as in the relapsing group, which is rather surprising. Those who were "cured" had only been ill, on the average, for a comparatively short time; however, as there was a great variation between the maximum and minimum duration of the

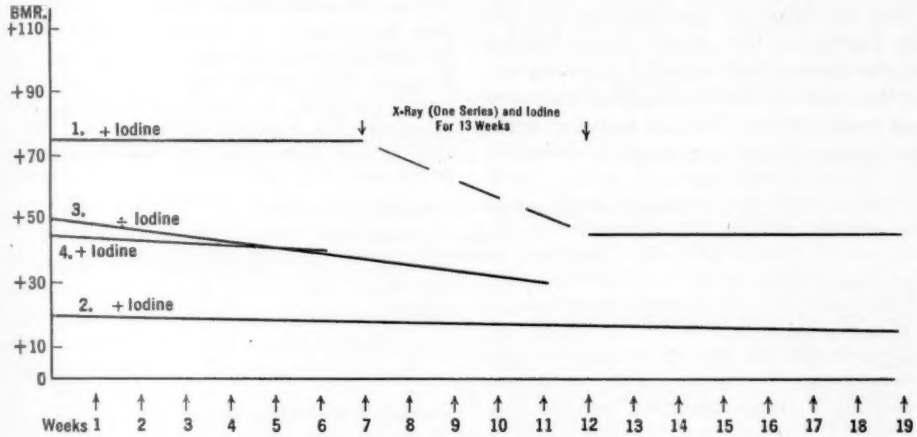

FIG. 2.—4 patients partly resistant to methylthiouracil treatment.

It appears from Table 4 that small to moderate-sized goiters have the highest percentages of cures, while the larger goiters and the nodular goiters show a smaller percentage of cures. Severity of the disease does not seem to have any influence on the results of therapy, but as was pointed out previously, the iodine-resistant cases do not do so well and constitute a separate group. As to sex, relatively more men fall into the "cured" group. In the age groups, there is clear-cut evidence that the younger and middle-aged groups have better results than the old-age group. This is somewhat discouraging as the latter are poor surgical risks due to increasing frequency of cardiac trouble. Even if good surgical risks, these people often state that they are too old for operation. As complications there were considered all the severe disease in each group, a comparison is only tentative.

The results correspond to those reported in the literature,[5,29,35] though Rose did point out that the complications were chiefly found in the relapsing group.

Of 38 patients, 33 were questioned closely on their ability to work. The average time from the beginning of methylthiouracil treatment until they had resumed full working capacity was 1.2 months.

During therapy we did not notice any significant increase in exophthalmus; on the contrary, in some cases the exophthalmus diminished.

The goiter only increased in size when the basal metabolism rate fell below normal.

Even though the patients were followed carefully during treatment some signs of

myxedema developed in 11 cases although the basal metabolism rate was around 0. In most cases the drug was withdrawn and first reassumed when the symptoms disappeared after 1 to 2 months of observation.

PARTLY INEFFECTIVE TREATMENT. Although quite a few authors frankly state that there are no methylthiouracil-resistant cases, 14 of these have been collected in the literature (Table 6), and Rose[35] has described 6 cases following thiouracil. From the reported case histories one has the impression that many of the failures are due to too short a period of treatment. In the cases of Rose *et al.*, the treatment was continued for 7 to 15 months; while the length of our treatment is shown in Figure 2. Cases 1 and 2 were certainly not improved in any way in spite of adequate doses. In Case 3 there was a slow fall in basal metabolism rate but no improvement in the general status of the patient. This also applies to Case 4, but we ought to have treated this patient for a longer period.

REACTIONS TO METHYLTHIOURACIL. As previously stated, several authors have stressed that the reactions depend upon the quantity of the drug. It is therefore fair to divide the material into 2 groups as in Table 5 in the same way as in Table 2. It is then very evident that the toxicity is much greater (78%) in the group receiving 1.2 gm. of methylthiouracil as an average initial daily dose than in those patients who received 0.57 gm.; the latter group had reactions in 47%. It is to be noted, however, that the only case of agranulocytosis occurred in the small dosage group.

A brief protocol of this case of agranulocytosis is as follows:

Case Reports. CASE 1. A 59 year old woman had had symptoms of thyrotoxicosis for 11 years which, from time to time, had been treated with iodine, with good results. She had the following complications: hypertension and thyrotoxic heart but without any arrythmias or cardiac insufficiency. Methylthiouracil, 0.6 gm., was given for 1 month with weekly control of blood and urine, but then, without any warning, the granulocytes suddenly disappeared for 7 days and at the same time she developed the usual accompanying symptoms. The drug was at once withdrawn, penicillin was instituted, and the patient recovered.

TABLE 5.—REACTIONS TO METHYLTHIOURACIL

	Iodine for a long period previous to treatment with drug	No long iodine period immediately prior to drug treatment	Totals
Maximum grams per day	1.20	0.57	
Average duration of treatment with high doses	28 days	20 days	
All—even slightest signs of reaction (% of group)	14 (78%)	20 (47%)	34 (56%)
Agranulocytosis	0	1	1
Neurologic symptoms (motor-sens.)	2	..	2
Fever	7	9	16
Skin eruption	5	8	13
Urobilinuria	12	7	19
Dyspepsia	6	8	14
Paresthesia	1	5	6
Vertigo	..	1	1
Leukopenia	1	2	3
Absolute lymphocytosis	..	1	1
Drug not reported	6	4	10
Repeated reaction after a small dose	2	3	5
No repeated reaction after drug	6	13	19
Transient myxedema during treatment	..	..	11

In the group not treated with iodine, the percentage of reactions (47%) while smaller than in the iodine group, was considerable, but in only 5 cases, one-fourth of this group, was it necessary, because of repeated drug reactions, to discontinue treatment.

The high incidence of reactions can be explained by the scrupulous questioning and examination of the patients at each visit. Actually some of the symptoms could have been attributed to intercurrent causes, but, when not proved otherwise, they were attributed to methylthiouracil,

One of the important findings was a positive urobilin test in the urine (the urine being diluted 10 times before the test) which often accompanied other signs of intoxication. When following these patients weekly they were always found to have a negative urobilin test until the signs of intoxication developed. We did not see any cases of jaundice, the blood bilirubin and the Takata-Ara test were normal when tried in connection with a positive urobilin test. Recently it was pointed out by Ehrich[13] that there is a rather high incidence of liver damage in experimental animals when treated with thiouracil and one of the first signs of sensitivity is manifested in the liver. In clinical reports this has hitherto not been mentioned, apart from the few cases where jaundice contributed to the toxic reaction.

The other reactions mentioned in Table 5 are in agreement with those reported by other investigators, but 1 case with neurologic signs must be mentioned separately (the other case was questionable). A brief protocol is as follows:

Case 2. A woman aged 64 had diabetes, slight hypertension and an adenoma of the thyroid. There were no signs of cardiac failure. She had been ill for 10 years and had often been hospitalized for diabetic coma and her diabetes was very difficult to control. The manifestations of thyrotoxicosis were insignificant and the diagnosis was only made when she was brought to our hospital, at which time she had a basal metabolism rate of +65. Iodine therapy was instituted, but she soon appeared to be iodine-resistant. As we did not know of thiouracil at that time, she was given a series of Roentgen ray exposures with continued iodine therapy. She remained unimproved and at the same time refused subtotal thyroidectomy. Following this, over a period of 4 months, she was given gradually increasing doses of methylthiouracil from 1 to 2.2 gm. a day, with very little effect (as we would now anticipate) except a slight reduction in the basal metabolism rate to +40. The doses were then gradually decreased to 0.8 gm. a day, yet slow improvement continued, and she now began to gain in weight, her diabetes was more easy to control, and she required less insulin. The patient was continued on 0.8 gm. a day and was thereafter treated as an ambulant patient. It was difficult to persuade her to report regularly, because of difficult transportation during the war, and 75 days after the last control observation, which was satisfactory, she came to the hospital in a serious condition. The first symptom she had complained of at home had been pain and difficulty in control of her left leg. On admission to the hospital, she looked somewhat myxedematous but her basal metabolism rate was only −1. At that time she could not walk at all. Objective examination showed paresis of the quadriceps on the left side but muscular strength was reduced in both legs and in the right arm. The patellar reflex was lost and the plantar reflex was diminished, acting in a peculiar, slow way. The Babinski sign was positive on the left side. There was ill-defined dysesthesia over the left thigh but no definite changes corresponding to the distribution of any peripheral cutaneous nerve. The spinal fluid showed no increase in cells, with albumin 30, and globulin 1. One month after the drug was withdrawn the patient began to walk and later did not have any disturbance of gait or other neurologic signs.

Survey of Reports on Methylthiouracil. Table 6 gives a survey of the literature on methylthiouracil treatment. There is a surprising difference between the various statements, the 2 largest series by Meulengracht[29] and Frisk[16] show extraordinarily good results. This may be due to some extent in the work of Frisk to his use of small doses and also because he does not give any details in his short summary. The percentage of sustained remissions in the whole material follows rather closely those seen in our cases, but in our patients the relapses were a little more common (20% as compared with 12%). Our figures on partly ineffective treatment are possibly too high, as explained earlier in this article. The number of discontinued treatments because of repeated reactions in our group which received the relatively small doses of methylthiouracil is a little higher (12% as against 7%), but the incidence of mild

TABLE 6.—REVIEW OF PUBLISHED DATA ON METHYLTHIOURACIL TREATMENT—A COMPARISON WITH THE RESULTS OF THIOURACIL

Authors	No. cases	Range of daily drug dose (gm.)				A Treatment effective						B Treatment partly ineffective	C Treatment discontinued because of reactions			Mild recurrent reactions not interfering with therapy
		Initial period		Maintenance period		Sustained remission after withdrawal	Average follow-up time after withdrawal	Preoperative treatment	Therapy discontinued not because of reaction	Reduction in drug successful, no attempt to withdraw	Remission after withdrawal		All cases	Agranulocytosis	Others	
Magnusson *et al.*	46	1.2	0.4	0.15	0.02	3	4 mos.	3	1	30	5	..	4	1	3	1
Mortensen *et al.*	17	1.8	1.0	..		1	5 mos.	6	..	..	..	1	2	1	1	3
Harsloef	12	1.0	0.8	0.2	0.1	1	3 mos.	..	..	..	..	2	..	..	..	
Ehlertsen	15	1.0	0.5	..		..	..	..	..	..	..	..	2	1	1	
Lundbaek	35	0.8	0.5	..		..	..	..	..	..	..	..	2	1†	1	4
Kristiansen	1	1.0		..		..	..	..	..	..	..	..	1	1		
Leschly-Jacobsen	4	0.8	0.6	..		..	..	..	..	..	..	..	4	1	3	1
Pindborg	10	0.8		..		..	..	..	..	..	..	..	1	..	1	1
Thyssen	28	0.8	0.6	0.2	0.1	..	..	5	..	..	..	2	..	..	..	4
Westergaard	22	1.2	1.0	..		..	..	9	1	..	..	4	..	..	..	3
Meulengracht	144	0.75		0.03		45	9½ mos.	10	..	75	5	..	7	..	7	5
Frisk	109	0.5	0.4	0.01	0.05	17	6 mos.	8	..	76	7	1	..	..	..	11
Harrestrup-Andersen	25	0.8		..		..	..	16	..	..	..	..	3	..	..	5
Vogt	17	0.8		0.2	0.05	..	..	..	..	..	..	2	3	2	1	5
Vogt Lorentsen	12	0.8		..		..	..	..	..	..	..	..	1	1	..	2
Wilson	30	1.0	0.6	0.1	0.025	3	12 mos.	9	..	..	16	2	..	..	..	2
Leys	16	1.0	0.8	0.1	0.05	..	..	1	1	..	..	..	..	..	..	2
This author	61	2.2	0.4	0.2	0.05	16	7½ mos.	4	2	10	12	4	13	1	12	21
Total	604	..		..		86	..	71	5	191	45	18	42	9	30	69
% of total cases						21	..	..	..	..	12	3	7	1.49	..	11
								THIOURACIL								
van Winkle	5745	..		..		..	..	..	..	..	..	4.3%	7.9%	2.50%	..	6%
Moore	1091	..		..		..	..	..	..	..	..	..	..	1.74%		

recurrent reactions is much larger than of any other authors.

A comparison of results of methylthiouracil treatment with those in a large series of thiouracil treatment collected by van Winkle *et al.*,[45] and Moore[30] shows about the same rate of partly ineffective treatment, discontinued treatment and agranulocytosis, whereas the mild recurrent reactions are less in the latter. This may be due to the fact that van Winkle's cases are collected from a number of clinics and not seen by the investigator personally.

At present it is impossible to make any comparison of methylthiouracil with propylthiouracil. It can only be pointed out that the effective doses seem to be the same.

Comment. It has been proved that methylthiouracil is a powerful antithyroid drug and it seems to exceed thiouracil in strength both in rat experiments and in human therapy. The difference is not great or significant.

A comparison with propylthiouracil is difficult, as the dosages of both drugs have yet to be stabilized, but it is clear that propyl- and methylthiouracil are to be administered in approximately equal doses. The earlier discrepancies between the rat experiments on the goitrogenic effect and the potency of the drugs in man are explained in a natural way by McGinty and by Christensen, and supply the reason for the failure of propylthiouracil to be as effective as would be expected.

Sensitivity to the drugs is even more difficult to compare, but thiouracil and methylthiouracil seem equal when one compares the fairly large series of methylthiouracil treatments collected by the Scandinavian and English investigators with the thiouracil series of Moore and van Winkle.

In our cases there is a high incidence of small but recurrent reactions. The reason for this lies not alone in the greater dosage used, because even in the small dosage group the incidence of reactions is high. Our attitude towards methylthiouracil was critical from the beginning because of the unphysiologic nature of the therapy and made us very alert and keen to find fault. Moreover, the many positive tests for urobilin constitute a considerable fraction of the so-called reactions. How much the reactions are due to allergy and how much to overdosage, it is impossible to say, but agranulocytosis especially seems to be a fairly late reaction, not caused by overdosage. There can be no doubt, from the common incidence of a positive urobilin test in association with other signs of reaction to methylthiouracil, that this test represents a toxic manifestation. The addition of this simple check-up is therefore advocated.

When using these drugs one has constantly to have in mind all the possible reactions to thiouracil compounds. We are dealing with drugs which cause reactions more often than do the sulfonamides. Moreover, both in the initial period and in maintenance therapy, we are using the drugs for a long period. The danger of agranulocytosis is constant, even if one uses propylthiouracil, following which such reactions have lately been observed by Shibley.[37]

Before radioactive iodine can come into common use, antithyroid drugs are a valuable form of therapy and the goal must be to reduce the dosage of the antithyroid substance as far as possible without diminishing its effectiveness and thus avoid reactions. The work of Danowski, Man and Winkle is very interesting in this direction and ought to be followed up, using methylthiouracil instead of thiourea; but it is quite possible that this procedure will not work in patients who are iodine-resistant, as they seem to constitute a special group.

Not only is it important to choose the correct minimal, initial dose, which we believe should not exceed 0.4 gm. a day, but it is also important to select the patients carefully to avoid unnecessary subtotal thyroidectomy which, even if the technique is perfect, has a mortality as high as 1.6% according to Wijnbladh.[41]

The percentage of complications (tetany and paralysis of the recurrent laryngeal nerve) is 2.8 and these figures are much higher when a second subtotal thyroidectomy is necessitated because of relapse (10 to 15%).[7]

From the standpoint of indications, antithyroid drugs have an important rôle in the treatment of cases which are: (1) too great a surgical risk or (2) in which operation is refused, or (3) which have had a relapse after a subtotal thyroidectomy. Unfortunately, many elderly patients belong to these 3 groups and as it has been shown, the very best results with methylthiouracil have not been reached in the elderly, as these patients do not have a sustained remission, but must continue on a maintenance dose. Another indication is (4) preoperative treatment. Methylthiouracil, together with iodine, for 10 days prior to operation, seems to lower the risk of a postoperative thyrotoxic crisis. A final (5) indication is constituted by those cases in whom one can expect "cure" from the treatment.

The percentage of "cures" differs greatly: reports[5,6,35,43] range from 20 to 82%, the mean being 54%. The reasons for this are: (1) the rather small material published; and (2) the rather short observation periods (only a few reports have an observation time longer than 9 months). Both in the literature and in our experience there is good evidence that a fairly high number of cases eventually do without therapy and remain well, especially in patients in whom the disease was not of long standing, the goiter comparatively small, and the patient in the younger age group. As Rose has pointed out, this group equals that group which, before the days of antithyroid drugs, one would expect to have had a self-limited course.

There is, on the other hand, no doubt that even the experienced worker has difficulty in predicting what course an individual case will run, and that prolonged iodine and Roentgen ray treatments have often brought the patients into trouble. Methylthiouracil is therefore clearly indicated in the above-mentioned cases. The maintenance therapy should not, however, be continued beyond 1 to 1½ years as it seems as if the chance of a sustained remission after this time is rather unlikely. At that time one ought to propose operation which would involve little risk, the patients not having had symptoms for 1 year.

Finally it is important to stress cases in which antithyroid drugs are contraindicated: (1) large intrathoracic goiter; (2) large adenomatous or diffuse goiter which is rapidly increasing; (3) in noncoöperative patients, where the control is difficult or even impossible; (4) thyrotoxicosis which is due to acromegaly (we have seen such a case which was resistant to methylthiouracil, as have Rose[35] and McGullagh[26]); (5) thyrotoxicosis in pregnancy is an unsettled matter. We have observed 1 case with a normal pregnancy and the birth of a normal child and so has Vogt,[46] but Frisk[16] has seen a child which was born with a pronounced exophthalmus. Freisleben and Kjerulf-Jensen[15] have shown that thiouracil passes the placenta in rats but that the newborn rats are normal. In the lactation period thiouracil is found in the milk and can do great harm to the offspring. The conclusion from this is that thiouracil should be avoided if possible in pregnancy and not used in the lactation period.

Summary. 1. In 61 patients with thyrotoxicosis so treated, methylthiouracil has been proved a potent antithyroid drug, but it causes reactions similar to those following thiouracil.

2. In 18 iodine-resistant cases of thyrotoxicosis treated with methylthiouracil, the basal metabolism rate fell much more slowly than is ordinarily the case.

3. Increasing the dosage of methylthiouracil beyond a certain high level is of no value.

4. To 18 patients large doses of methylthiouracil were given. The average dose was 1.2 gm. daily and the drug had to be discontinued in 8 cases (44%) and caused reactions in 14 patients (78%). A smaller

average dose of 0.57 gm. was given to 43 patients and in these patients the drug had to be discontinued in 5 cases (12%) and caused reactions in 20 cases (47%). In this group of patients there occurred a case of agranulocytosis which recovered.

5. The largest single dose recommended is 0.4 gm. a day, and even if this does not give any results for the first 7 or 8 weeks it is justifiable to wait 2 or 3 weeks more for a fall in basal metabolism rate.

6. Of 61 patients with thyrotoxicosis it was possible to carry the treatment through in 44 patients and they were fully capable of work in an average of 1.2 months after therapy was started.

7. Sustained remission with an average observation period of 7½ months occurred in 16 patients (26.2%) after a treatment of approximately 5 months' duration.

8. The sustained remissions occurred most frequently in cases with a small to moderate-sized goiter, in the young or middle-aged groups, especially in men, and in cases where the disease had not been of long standing. The complications and the severity seemed to have no significant influence on the ultimate result of therapy.

9. In 4 cases of thyrotoxicosis, methylthiouracil treatment, even for a long period, was unsuccessful.

10. Among reactions to methylthiouracil a positive test for urobilin in the urine is shown to be common and important.

11. A survey of the literature or methylthiouracil has been made and the results are compared with those of thiouracil and propylthiouracil. Methylthiouracil seems more effective than thiouracil and about as effective as propylthiouracil.

12. Indications for therapy with antithyroid drugs are outlined.

13. Methylthiouracil is of no use in elevated basal metabolism rate caused by acromegaly.

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THE EFFECT OF LUMBODORSAL SPLANCHNICECTOMY ON THE BLOOD VOLUME AND "THIOCYANATE SPACE" OF PATIENTS WITH ESSENTIAL HYPERTENSION

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This investigation was undertaken as part of a comprehensive study of the effects of sympathectomy on the hemodynamic functions of patients with essential hypertension. It was considered worthwhile to rule out the possibility that the reduction in blood pressure is accompanied by significant alterations of total blood volume, and, incidentally, to obtain information on the effects of the operation on the intravascular and extracellular fluid compartments of the body.

Following sympathectomy in normal cats the plasma volume increased,[11] whereas, in dogs with experimental renal hypertension sympathectomy produced either no change[4] or a decrease in red cell volume.[16] The present report presents the results of blood volume and "available fluid" ("thiocyanate space") studies in patients with essential hypertension before and after lumbodorsal splanchnicectomy.

Materials and Methods. The subjects were a heterogeneous group of 10 patients with essential hypertension. The measurements of plasma volume using the dye T-1824, and of "available fluid" volume using a 5% solution of sodium thiocyanate intravenously were determined before operation and at various intervals after operation.

The method of Gregersen and Stewart[6] for the simultaneous determination of blood volume and "thiocyanate space" was modified for use with the Coleman junior spectrophotometer. The blood samples were collected in 4 cc. hematocrit tubes containing a measured amount of liquid oxalate mixture in isotonic saline as described by Emerson and Ebert.[2]

The plasma specific gravity of each sample was determined with the Barbour and Hamilton falling drop apparatus and the dyed plasma samples were corrected for fluid shifts as recommended by Gregersen.[7] After such correction straight line dye concentration curves were obtained on a semi-log plot (logarithm of the plasma dye concentration plotted against time). Using this method uniform rates of dye disappearance (approximately 5% in the 1st hour) were obtained, thus confirming the observations of Noble and Gregersen.[14] For this reason and because of the greater convenience we determined the plasma volume from the 10 minute sample in the latter half of this investigation.

Since in some individuals thiocyanate reaches an equilibrium with the extracellular fluid slowly, and because there is no appreciable change in concentration from the 1st to the 4th hour following injection,[1] the plasma samples used to calculate the "available fluid" volume were drawn 1½ to 2 hours following injection. In view of the lack of adequate information concerning the precise volume distribution of thiocyanate "correction" factors were not used. The "available fluid" volume as used in this study expresses the total volume in which thiocyanate is distributed, including the plasma volume, the amount that enters the red cell, and its distribution in the extracellular space.

TABLE 1.—BLOOD VOLUME AND AVAILABLE (THIOCYANATE) FLUID CHANGES FOLLOWING LUMBODORSAL SPLANCHNICECTOMY.

Patient	Sex	Age	Time	Height (cm.)	Weight (kilo)	Blood pressure (mm. Hg)	Plasma volume (cc.)	Hematocrit (%)	Total blood volume (cc.)	Change (%)	"Available fluid" (cc.)	Change (%)
J. F.	M	25	Preoperative	175	80	155/100	2860	50.5	5720	...	20,500	
			P. 1st stage 10 days		77	170/135	3110	45.0	5650	− 1	20,600	0
			P. 2nd stage 12 days		76	135/95	2930	47.5	5610	− 2	24,100	+23
			3 months postoperative		78	120/85	2750	43.0	4880	−14	21,600	+10
			6 months postoperative		83	145/100	2930	47.5	5610	− 1.5	21,250	+ 9
E. L.	F	21	Preoperative	162	39	225/150	1575	40.5	2650	...	11,900	
			P. 1st stage 8 days		39	170/100	1880	33.0	2950	+11	13,800	+16
			P. 2nd stage 10 days		38	200/110	1680	39.0	2750	+ 5	13,100	+13
			3 months postoperative		40	115/88	2000	40.1	3350	+25	12,700	+ 8
			6 months postoperative		38	145/125	...	38.0	...	...	12,000	0
E. D.	M	35	Preoperative	176	77	165/135	3240	45.5	5950	...	20,200	
			P. 2nd stage 12 days		72	170/120	3000	37.5	4800	−16	21,800	+16
			3 months postoperative		68	115/88	2900	42.5	5050	−10	18,200	−10
			6 months postoperative		77	135/95	3350	43.1	5900	− 1	19,600	− 3
J. Mc.	M	42	Preoperative	170	74	165/110	2760	45.5	5070	...	18,000	
			P. 2nd stage 8 days		70	130/90	3210	38.5	5140	+ 5	22,600	+28
			3 months postoperative		72	145/100	3.00	33.0	5000	− 1	16,400	− 9
			6 months postoperative		72	130/100	2 40	42.7	4950	− 2	18,000	0
R. D.	M	27	Preoperative	181	64	220/140	3420	40.8	5970	...	19,200	
			P. 1st stage 5 days		63	220/155	3250	37.0	5170	−10	19,500	+ 2
			Immediate P. 2nd stage		...	160/130	3370	33.0	5430	− 6	21,800	+18
			P. 2nd stage 12 days		62	185/140	3570	37.0	5680	− 1	20,400	+ 6
			6 months postoperative		65	195/155	3520	43.5	6230	+ 4	20,400	+ 6
W. R.	M	33	Preoperative	177	77	190/135	2360	43.0	5125	...	18,200	
			P. 2nd stage 10 days		72	160/140	2220	51.0	4525	− 9	19,200	+13
J. S.	F	44	Preoperative	151	57	205/105	2300	40.0	3840	...	14,300	
			P. 1st stage 5 days		57	140/86	2520	32.5	3740	− 3	13,800	− 3
			P. 1st stage 10 days		57	160/95	23.0	37.0	3375	− 5		
			P. 2nd stage 15 days		57	165/110	2200	39.0	3320	− 6	14,700	+ 3
J. V.	M	35	Preoperative	178	86	160/120	2 40	43.8	5540	...	20,115	
			P. 2nd stage 5 days		84	158/110	2760	44.5	4975	− 9	24,300	+24
			2 months postoperative		85	135/112	...	47.5				
J. W.	M	42	Preoperative	170	80	165/110	3210	44.7	5800	...	19,700	
			P. 1st stage 10 days		77	210/110	3 50	43.5	5570	− 4	22,600	+15
			P. 2nd stage 20 days		73	140/93	3000	40.2	5000	−14	21,730	+22
C. C.	M	45	Preoperative	175	65	210/130	2550	53.0	5420			
			P. 2nd stage 10 days		65	170/110	2530	50.5	5170	− 5		

RESULTS. The results listed in Table 1 and Figures 1 and 2 reveal a decrease in total blood volume of from 1 to 16% in 9 of the 10 subjects during the convalescent postoperative period. In most cases this was due to a decrease in red cell volume as manifested by a fall in hematocrit. These observations were made about 10 days postoperatively in order that the results would not be confused by the

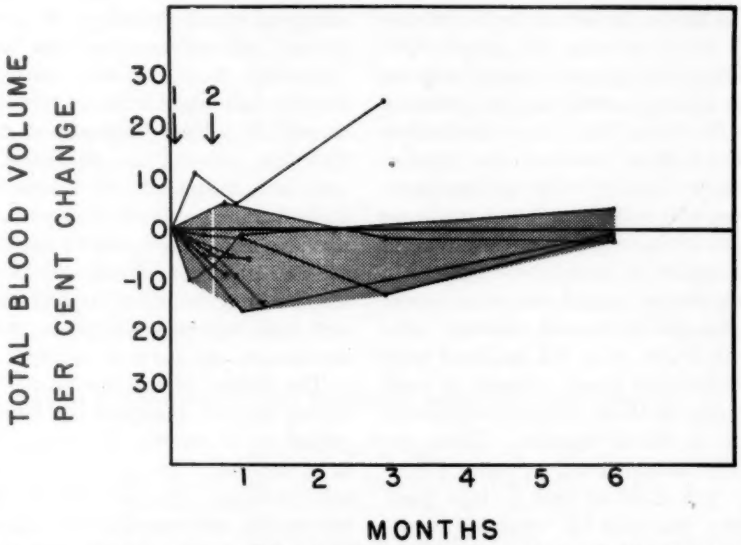

FIG. 1.—Per cent changes in total blood volume following lumbodorsal splanchnicectomy. The shaded area contains the values found in 9 of the 10 subjects studied. The arrows indicate the times of operation.

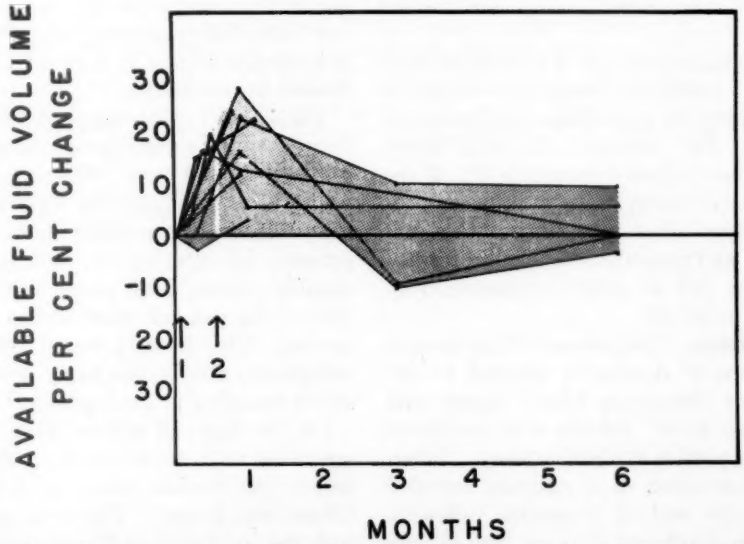

FIG. 2.—Per cent changes in "available fluid" volume following lumbodorsal splanchnicectomy. The shaded area contains the values found in the 9 subjects studied. The arrows indicate the times of operation.

routine administration of blood and other parenteral fluids during the operative and immediate postoperative periods.

Coincident with the fall in total blood volume there was an increase in "available fluid" in all of the 9 cases studied varying from 3 to 28%. In all of the 5 patients followed for 6 months the total blood volume and "thiocyanate space" was restored to approximately the preoperative levels at the end of the period of observation. In 4 of these, however, the hematocrit remained slightly below the preoperative value, the deficiency being made up by a slight increase in plasma volume.

The decrease in total blood volume in the postoperative period has no relationship to changes in arterial pressure. Patients with slight or no fall in blood pressure exhibited the same decrease in total blood volume as those who had significant reductions in blood pressure. Thus, patient R. D. whose blood pressure preoperatively was 220/140 and 5 days postoperatively was 220/155 revealed a decrease in total blood volume of 10%. Patient J. S. whose blood pressure fell significantly from 208/105 preoperatively to 160/95 10 days postoperatively also exhibited a decrease in total blood volume of 5%.

Similarly, at the end of 6 months there was no correlation between changes in arterial pressure and changes in total blood volume. For example, the total blood volume had returned to within 2% of the preoperative value in both patient J. F. whose blood pressure was essentially unchanged and patient J. Mc. who exhibited a definite fall in arterial pressure from 165/135 to 135/95.

Discussion. The purpose of this investigation was to determine whether or not significant changes in blood volume and "available fluid" volume were produced following partial sympathectomy. Long-term observation of 5 patients revealed that at the end of 6 months following operation significant changes had not occurred, despite the fact that in the majority of these cases the blood pressure was significantly lower than the preoperative value.

However, during the course of the study definite trends in blood and available fluid volume were noted in the period from 8 days to 2 weeks after operation. These changes, which consisted of a reduction in red cell volume and an increase in "available fluid" volume, have been observed following other types of major surgery.[13] The fluctuations observed, therefore, cannot be considered to be a specific consequence of sympathectomy. Indeed, an increase in "thiocyanate space" has been observed in other conditions, such as lobar pneumonia[15] and infectious hepatitis,[12] as well as after surgical operations, and may represent simply a non-specific reaction of the body to injury.[17]

The failure of the hematocrit value to return to the preoperative level over a period of 6 months following operation is of interest. In dogs with experimentally induced polycythemia Schafer[16] has observed a definite reduction in red blood count following sympathectomy. He also noted a fall in hematocrit in a patient with polycythemia rubra vera who had undergone sympathectomy. Green[5] has reported a marked reduction in the hematocrit value following surgical excision of a pheochromocytoma in a patient who had chronic hypertension.

These observations suggest a relationship between the sympathetic nervous system and the red blood cell volume. However, the change may be more apparent than real, as it is possible that sympathectomy, by altering the caliber of the smaller vessels, may cause a redistribution of the red cell mass in the vascular system. This in turn would change the cell-plasma ratio in the larger vessels from which sampling is accomplished.[3,8,19]

On the basis of surface area the preoperative total blood volume values were within the normal range as defined by Gibson and Evans.[9] This is in agreement with the conclusion of Harris and Gibson[10] that the blood volume is normal in essential hypertension. The values for "avail-

able fluid" volume similarly fell within the normal range when compared on the basis of surface area.[18]

Summary and Conclusions. 1. Repeated blood and "available fluid" determinations were made on a series of 10 cases of essential hypertension for periods up to 6 months following lumbodorsal splanchnicectomy.

2. The total blood volume was reduced in 9 cases in the period from 8 days to 2 weeks following operation, due primarily to a diminution in red cell volume. Despite a continued reduction of blood pressure the total blood volume was restored to the approximate preoperative level at the end of 6 months following operation. A slight reduction of the hematocrit value persisted, the deficiency being made up by a compensatory increase in plasma volume.

3. Coincident with the reduction in blood volume noted in the 2nd postoperative week there was an increase in "available fluid" volume. This elevated value was restored to the preoperative level over a period of several months.

4. Previous observations that the total blood volume is within normal limits in patients with essential hypertension were confirmed.

5. In relation to surface area the "available fluid" volume of hypertensive subjects was also found to be within the normal range.

6. The reduction in blood pressure following sympathectomy is not dependent upon changes in total blood or "available fluid" volume.

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FLUORESCEIN STUDIES IN PERIPHERAL VASCULAR DISORDERS

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Although there is a number of procedures which are now employed to determine the status of the peripheral circulation, any method which may add to our knowledge or promise more information is desirable. Contributions and reports on "circulation time" which indicated blood flow seemed to offer some encouragement. These studies were based on arm to tongue and arm to lung time, whereby one could identify whether the right heart or the left heart was involved or which one was predominating.

More recently fluorescein was used in determining circulation time. It was given intravenously and investigators claimed that this method was an improvement over the other substances because it eliminated personal equation of the patient, and the end point could readily be seen by the observer by the appearance of the dye upon the lips or the conjunctiva.[2,4] Furthermore, fluorescein was particularly preferable when the patient could not cooperate by calling the end point for the circulation time.

On a previous occasion, one of us attempted to investigate the circulation time in the extremities, both upper and lower. A 20% solution of calcium gluconate was used. The results at that time were vague and unsatisfactory, and our series was not extensive enough to warrant publication. Our conclusions concurred with those of Berk[1] who used magnesium sulfate. His results were likewise unsatisfactory because of the large percentage of "blanks"; that is, where no sensation of warmth was felt in the peripheral parts. He noted that "two-thirds of all the individuals showed blanks at some time or other."

Our hopes were encouraged when Lange[4,5] published his results of fluorescein in a patient with embolic occlusion and one of diabetic gangrene. We considered that fluorescein, with its advantages over other substances commonly employed for circulation time, offered more favorable results.

Method of Study—Technique. After the patient has rested for about 15 to 20 minutes, he is placed on a table in the recumbent position. The limbs are exposed and inspected preliminarily with an ultraviolet bulb covered with a purple glass filter. This is to search for any abnormalities and to acquaint oneself with the color and condition of the extremities.

Under the usual sterile precautions an 18 gauge needle is inserted into the antecubital vein.

The room is then darkened and 4 cc. of fluorescein solution (20%)* is rapidly injected (3 cc. was used at first but later 4 cc. of the solution was used in the remaining cases).

The examiner, with stop-watch in hand, observed the lips which are the first to show the end point of the distribution of the dye. A greenish or greenish-yellow color can clearly be seen. Then the hands and lower extremities are carefully scrutinized and the time required for the distribution of the dye to these parts is recorded. In our study we charted (1) the elbow to lip time, (2) elbow to hand time and (3) elbow to foot time.

Selection of Cases. A series of 89 patients were studied, 69 of which were analyzed for statistical purposes. Forty-five patients had some form of vascular disorder ranging from arteriosclerosis obliterans, thrombo-angiitis obliterans and embolic occlusion to Raynaud's syndrome, chronic

* Fluorescein supplied by courtesy of Eli Lilly & Co., Indianapolis, Indiana.

leg ulcers and vasospastic disorders. They were taken consecutively from the vascular clinic.

Another series of 24 patients were similarly studied; they were obtained from the medical and surgical wards. Some were convalescing from operations or were admitted for study. The medical cases included cardiac and miscellaneous conditions. However, these cases were presumably not suffering from any peripheral vascular disorder. They were of various ages, color and sex.

TABLE 1—APPEARANCE TIME

Peripheral Vascular Disease Group

	Times (sec.)	Average (sec.)
Arm to lips	12–30	16.0
Arm to hand	15–51	31.2
Arm to foot	30–120	61.4

Non-vascular Disease Group

	Times (sec.)	Average (sec.)
Arm to lips	7–25	14.4
Arm to hand	17–60	28.5
Arm to foot	26–60	53.4

Results and Discussion. Table 1 shows the appearance time of the fluorescein on lips, hands and feet in the vascular group and those patients who presumably were not suffering from peripheral vascular disorders.

Arm to Lip Times. Twenty-two of the combined group showed an appearance time from 8 to 13 seconds, while 47 revealed a circulation time ranging from 14 to 25 seconds. These results seem to compare satisfactorily with Lange's figures.[6] He noted that in studying 89 normal adults above the age of 20 years, the appearance time at the lips was between 15 to 20 seconds, an average of 17.1. Fishback's[2] figures were from 15.6 to 17 seconds; he gave no average in his series. Our average was 14.4 to 16 seconds.

Arm to Hand Time. Thirty-four cases showed an appearance time between 17 and 28 seconds (49.3%); 17 of the combined group revealed the dye between 29 and 35 seconds (24.6%). Collectively 51 of the patients studied (74%) showed the dye in the hands between 17 and 35 seconds. Our average for the non-vascular cases was 28.5 seconds and 31.2 seconds for the peripheral vascular group.

These figures have been arranged in Table 2 showing the comparison with those obtained by Berk,[1] whose averages were 19.2 seconds for normals and 25.1 seconds for the vascular disease group.

TABLE 2—ARM TO HAND TIME

Fluorescein

	Appearance time (sec.)	Average (sec.)
Non-vascular	17–60	28.5
Vascular	15–51	31.2

Magnesium Sulfate (Berk)

	End point (sec.)	Average (sec.)
Non-vascular	6–61	19.2
Vascular	12–60	25.1

Table 2 shows the comparative results of the arm to hand time using fluorescein and magnesium sulfate. Twelve of the patients revealed a prolonged time between 41 to 60 seconds, 10 being in the vascular series and 2 in the non-vascular. He believes that the delayed appearance of the dye could be explained on the basis of age and cardiac disease. However, some of them might have had a variable degree of interference with the circulation as well.

Arm to Foot Time. Almost invariably the appearance of fluorescein was delayed in the lower extremities. It was first seen on the lips, then on the hands, and last on the feet. Only in 2 or 3 cases did the dye appear in the feet as promptly as it was seen in the hands. Its late appearance in the feet is quite understandable when we take into consideration that the skin temperatures are always lower in the feet than in the upper extremities. It is also an accepted fact that the vasoconstrictor gradient is greatest in the feet. It may be noted that 6 cases in the non-vascular group (25%) showed the dye between 30 to 40 seconds in comparison with 6 cases in the vascular group (17.8%); 11 cases of the non-vascular series (46%) showed an appearance time between 30 to 50 seconds compared with 15 cases of the vascular series (33%). The analysis of the

arm to foot time in these cases was carried out to a 3 minute period. It was noted in the cases that showed a markedly delayed appearance time over 50 seconds, that this occurred in 43.5% of the vascular cases as compared with 18.2% of the non-vascular cases or about 2.5 to 1. These facts we believe are significant and indicate that vascular disorders can be responsible for the prolonged appearance time. The delayed end point of the fluorescein in our series of the vascular group was probably due to the disturbance in the circulatory system. However, a few of these patients may have had some cardiac condition as well but we are reasonably certain that the age element was eliminated.

Untoward Effects. Our experience compares with other investigators who used fluorescein in their studies. There were no definite reactions. Two of our patients vomited shortly after the test but this, we feel, was due more to nervousness and apprehension than to the effect of the dye. Over 100 injections were given in our studies. The dye was rapidly eliminated by the kidneys. The patients were told to expect a greenish tinge on the skin to remain for a few hours and that the urine would be discolored temporarily.

Normals. We realize that before any definite conclusions can be reached in reference to the employment of fluorescein for investigating the peripheral circulation, one must have a fairly acceptable normal appearance time. Because of the scarcity of investigations with this substance, we were handicapped in determining what the normal standard is. For the benefit of other investigators in the future we would suggest the following figures. These are based on our observations with corrections which we suggest that would make them approximate of what may presumably be normal. This will particularly apply to the arm to foot time which will be more than the other regions.

TABLE 3. NORMAL FLUORESCEIN APPEARANCE TIME

Arm to lip time	12–16 seconds
Arm to hand time	18–28 seconds
Arm to foot time	40–50 seconds

These figures are higher than those quoted by Berk in his studies of the circulation time in the lower extremities using magnesium sulfate. In his normal group he noted that the end point ranged anywhere from 7.4 to 8.2 seconds to 50.1 to 56.2 seconds, an average of 24.1 seconds. In the pathologic series, he recorded 18 to 70 to 75 seconds, averaging 35.7 to 36.1 seconds. His figures are quite at variance with those suggested by our studies. However, we feel that it is impossible to arrange any standard figures for those patients with peripheral vascular disease.

However, there is another fact which deserves some comment at this time and which may suggest an earlier normal appearance time than the figures quoted by us. Lange[5] employed fluorescein in 4 patients who had ulcers on the lower extremities. He specified that these ulcers were not arteriosclerotic in type and we presume that the patients were not cardiacs. Fluorescein was seen in the ulcer areas within 23 seconds.

Neller and Schmidt[8] employed fluorescein by making a series of superficial scratches at 2 inch intervals down the legs and observing the appearance of the dye in these areas as a means of determining the level of vascular impairment. However, they do not quote any normal values of appearance time.

Whether the dye may be seen more promptly in an ulcer area than working its way through the peripheral arterioles and capillaries is a matter which we are not prepared to settle at this time.

It may be of interest at this point to comment on some features which are demonstrated by the dye technique. In 1 patient we were impressed with appearance of "blacked-out" areas which on physical examination were rose-colored. These so-called rose spots were described in a previous publication[3] as evidence of early pathologic damages in the smaller vessels. While we were unable to prove this fact by histologic section we are now convinced that these "blacked out" areas

are due to interference with the circulation. This is in accordance with Lange's views. A second feature was the application of fluorescein in studying the condition of ulcers when skin grafting is considered. Lange[7] pointed out that he was able to select those ulcers with healthy granulations and those which showed definite evidence of interference with blood supply by the use of fluorescein, this distinction not being apparent on ordinary inspection. A third feature which interested us when examining a patient who had a unilateral lumbar ganglionectomy: the appearance time was practically the same in each foot, but the dye was more dense on that side which was operated upon despite the fact that it had a greater degree of involvement. Feature No. 4 was apparent in a patient who had an arteriovenous fistula in the groin. After 3 attempts to obliterate this condition, the patient still had considerable pain and the foot was cold and discolored. On examination with fluorescein the foot and ankle areas were practically "blacked-out" indicating extensive interference with the arterial blood supply.

Summary and Conclusions. Eighty-nine patients were studied with fluorescein for the appearance time at the lips, hands and feet. Sixty-five of this series had some form of vascular disease, 24 cases were presumably not vascular. They were various ages and sex.

The appearance time in the lips in the non-vascular group was 14.4 seconds and 16 seconds for the patients with vascular disorders.

The arm to hand time showed an average of 28.5 seconds for the non-vascular cases and 31.2 seconds for the vascular group.

The arm to foot time ranged from 30 seconds to 3 minutes. However, most of the cases showed an appearance time within 60 seconds, the average for the non-vascular group was 53.4 seconds and 61.4 for the vascular cases. When the appearance time was beyond 60 seconds, the vascular series predominated over the non-vascular about 2.5 to 1.

Other features which may explain a delayed appearance time are old age and cardiac disease. The appearance time is not so easily recognized in the lower extremities and in the colored.

The following figures are suggested for the normal appearance time: arm to lip, 12 to 16 seconds; arm to hand, 18 to 28 seconds; arm to foot, 26 to 60 seconds (average 53.4).

The fluorescein test is superior to other substances which depend on subjective sensations for the end point. The end point with this method is readily recognized. The fluorescein test is harmless and non-toxic, the dye is readily eliminated by the kidneys.

Despite the unavailability of a more definite normal standard for arm to foot time, we were impressed with the delay in appearance time in the lower extremities among patients with known vascular disorders. From this, we infer that it gives information relative to the status of the peripheral circulation. It is helpful in deciding and selecting the proper type of ulcer for grafting. It may be of assistance in determining the level for amputation, but we have had no personal experience with fluorescein along these lines.

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THE ANTIDIURETIC EFFECT OF MORPHINE AND DEMEROL IN CONGESTIVE HEART FAILURE*

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Antidiuretic action of morphine has been studied in animals since 1887.[27,30,36] Frey,[18,19] in 1907, concluded that narcosis with morphine hinders the development of a water diuresis. During the next 20 years studies on the water diuresis in animals[16,17,21,32,34,35] continued to confirm this fact. Bonsmann[3,4] and others,[15,33] doing a more careful control than previous workers on the initial state of hydration of the animals, reëmphasized the antidiuretic effect of morphine. De Bodo and coworkers, working from 1940 to 1945,[11,12,13] have presented detailed pharmacologic studies made in animals that clearly demonstrate the antidiuretic action of morphine.

The antidiuretic action of various hypnotics has been studied but the results are controversial.[7,24,26,28] Recently de Bodo has shown in animals[13] that some barbiturates have an antidiuretic action.

The effect of drugs on mercurial diuresis has also been studied in animals. This work includes studies of the antidiuretic action of barbiturates,[5,38] chloralose,[5] pituitrin[37] and antipyretics.[1]

In summary, the experimental work in animals has shown that morphine inhibits water diuresis. Whether hypnotics exert a similar effect on water diuresis is controversial. Hypnotics, antipyretics and pituitrin have been shown to exert an antidiuretic effect on mercurial diuresis. This present study concerns itself with the action of morphine on mercurial diuresis.

In man the antidiuretic effect of morphine and allied compounds has long been a clinical impression. It was probably first mentioned in the literature in 1839 when Bouchardat[6] reported the alleviation of the symptoms of diabetes by gradually increasing doses of opium. The report of decreasing urinary volume in diabetes mellitus and diabetes insipidus following morphine has been repeatedly reported since then. Recently, Master has reported as his clinical impression that morphine reduces the effect of mercurial diuretics.[25] The study by Fee[16] using himself as the subject on the inhibition of water diuresis by morphine constituted the first controlled clinical investigation. The clinical studies presented by Hopmann,[23] however, yielded irregular results. His patients were in various stages of digitalization, were not studied under standardized conditions, and were victims of additional diseases such as cirrhosis and nephritis. He stressed morphine as a diuretic. In those instances where it failed to act as such, he explains this failure to initiate diuresis by stating that oral morphine given at night produces diuresis and morphine given during the day does not. Bahn, Iserbeck and Lindemann,[2] on the other hand, presented a controlled clinical series of observations and noted definite decrease in water diuresis following morphine. However, the work of Hopmann served to impress certain workers to such an extent that as late as 1940 a review of recent advances in therapy[8] carried the statement that morphine alone may cause massive diuresis

* Presented in part before the New York Heart Association Scientific Meeting, May 10, 1946.

in cardiac patients, although it is accepted as an antidiuretic in normal individuals.

Although it might be anticipated from the literature[7,12,17,18] that morphine and demerol would exert no consistent antidiuretic effect on saline diuresis, the frequent use of mercurial diuretics and these drugs during the treatment of cardiac failure makes the study of their interaction a matter of some importance.

Method. The study was divided into 2 parts: the action of morphine on diuresis in patients with congestive heart failure, and in normal persons. In patients with heart disease the diuresis was initiated as a result of mercupurin. The study in normal persons was made during water diuresis. All patients studied were in the fasting state. The urine samples were obtained by means of a catheter. The bladder was first emptied and the sample discarded. A preliminary determination of the rate of urinary flow was made during the next 20 minute period, by measuring the volume excreted in these 20 minutes and expressing the urinary flow as cc. per minute. This sample of urine was saved for an analysis of chloride content. Subsequent samples were collected every 20 minutes. In both groups studied, the urine flow during the initial control period remained under 1 cc. per minute. When the urine flow rose above 1 to 1.5 cc. per minute, diuresis was considered to be initiated. The following technique was used to insure complete emptying of the bladder at the end of each 20 minute interval: Pressure over the pubis caused the expelling of the larger proportion of the urine. Twenty to 40 cc. of air was then instilled into the bladder by means of a syringe. If any urine remained, when pubic pressure was again instituted, the urine was expelled ahead of the air, which followed as bubbles. If the bladder was empty the air alone was expelled at once, and with a characteristic explosive sound. This end point was sought at least twice at the end of each period. The concentration of chloride in the urine (expressed as milliequivalents per liter) was measured in each sample by the method of Schales and Schales.[31] The rate of chloride excretion (milliequivalents per minute) was calculated from the urine flow (cc. per minute) and the chloride concentration (milliequivalents per liter).

Study of the Antidiuretic Action of Morphine and Demerol in Cardiacs. All subjects studied were patients who remained in congestive heart failure despite complete bed rest and full digitalization. All patients suffered from the type of cardiac decompensation in which frequent mercupurin injections were needed to mobilize edema. Each patient received 3 gm. of ammonium chloride daily and a low fluid, salt-poor diet. As soon as daily weights were stabilized on bed rest, mercupurin was given in each case in order to initiate diuresis. In order to establish the type of response obtained with mercupurin alone, control diuretic curves were obtained on a group of 6 patients, each of whom received 2 cc. of mercupurin intravenously. Nine observations were made on these 6 patients. One patient was studied 3 times, 1 patient twice, and 4 patients were each studied once. Nine patients were then studied to determine the effect of morphine on mercurial diuresis. Two cc. of mercupurin were given intravenously and when diuresis was well established the patient received 0.01 gm. of morphine sulfate intramuscularly—usually about 80 minutes after the injection of mercupurin. The urine flow was followed for 3 to 5 hours after mercupurin. The patient was then allowed to reaccumulate edema to the original weight level, and 2 cc. of mercupurin alone were given. Thus, a control diuretic curve with which to compare the curve under the influence of morphine was obtained on each patient.

Study of the Antidiuretic Action of Morphine in Normals. Water diuresis studies were made on 4 patients on the wards of Bellevue Hospital. They were all awaiting discharge. Two were convalescing from upper respiratory infections (la grippe, bronchitis) and 2 were victims of mild nervous system disease. All 4 were free of circulatory, renal or hepatic disease. In accordance with De Bodo's technique in animal studies, the 4 patients were completely hydrated before the ob-

servations were begun. This was accomplished by the administration, in the fasting state, of 1000 cc. of water by mouth at 7 A.M. of the day of the study. This water of hydration was excreted in varying amounts over the next 3 hours, depending on the original state of hydration of each subject. Three hours after the first ingestion of water, at 10 A.M., the study was begun. Urine samples were obtained every 20 minutes for 2½ to 3 hours by the same catheter technique as above. It is known that if a person is completely hydrated, he will excrete almost quantitatively a 1000 cc. of water in 2 to 3 hours. The 4 patients studied demonstrated this clearly in the control observations to which each was subjected. Three of the patients received the second dose of fluid as 1000 cc. of 5% glucose in distilled water, intravenously, given in 45, 40 and 17 minutes respectively. One patient received the second 1000 cc. of water by mouth, given in 5 minutes.

Results. *Cardiac Patients.* The control diuretic curves obtained show in general an early rise in urine flow during the first 3 to 5 hours following the injection of mercupurin. From a normal level of under 1 cc. per minute, the urine flow rose to a minimum level of 2.4 cc. per minute in 1 patient to a maximum level of 10 to 12 cc. per minute in others. The peak of the curve was reached during the first 1 to 2 hours and then the diuretic curve leveled off and was maintained fairly constantly at a plateau range for the next 2 to 3 hours. Chloride concentrations, and therefore chloride excretion rates, behaved in the same manner. In a patient with several diuretic curves, the chloride concentration rose to almost exactly the same level on each trial and remained at this level with a variation of only 10 to 15 milliequivalents per liter.

Nine patients in congestive heart failure were studied to note any effects by morphine on the diuretic curve. In 3 patients morphine was found to have an antidiuretic effect. The latter was interpreted on the basis of a persistent depression of the diuretic curve obtained during the period of observation and in each patient compared with his own control diuretic curve.

It should be emphasized that in the same patient at the same weight level the diuretic curve follows the same pattern on repeated diuretic trials and the control curves do not show a fall in rate of urine flow during the first 3 to 4 hours.

The first patient (C. P.) was studied repeatedly. In 2 separate observations, each with 1 or 2 control curves, morphine, when administered after the diuresis was in progress, caused a definite suppression of this diuresis (Figs. 1 and 2). This was first noted 40 minutes after the administration of the drug and persisted for as long as 2½ hours. The urine flow fell from 10.3 cc. and 7.4 cc. per minute to 2.35 cc. and 3.7 cc. per minute respectively, and then gradually rose to 7.75 cc. and 5.8 cc. per minute at the end of the period of study. In a third observation, when morphine was given 10 minutes before mercupurin, *i. e.*, before the diuresis developed, the diuretic curve was flattened and ranged between 1.47 and 5.14 cc. per minute and had not risen to the control level, 8.5 cc. per minute, 4 hours after morphine was given (Fig. 3). As an additional control in this patient, a sterile water injection was given intramuscularly after the development of the diuretic state but the diuretic curve was unaffected (Fig. 2). The intramuscular injection of 75 mg. of demerol after the diuresis had started produced in this same patient the same antidiuretic effect as morphine and caused a slight decrease in urine chloride concentration (Fig. 4). However, the effect was noted sooner (24 minutes after demerol was given). The chloride concentration in the urine differed very little from the control levels during the antidiuretic effect of both these drugs. Therefore, the chloride excretion rate varied only with water output or urine flow. The total chloride excretion during the period studied was markedly reduced under the influence of morphine and

demerol as compared with the control periods. Control total chloride excretion over 4 hours ranged between 219 and 314 milliequivalents and during antidiuresis fell to a range between 103 and 171 milliequivalents (see Table 1).

The second patient (S. W.) was also studied repeatedly. An antidiuretic effect was also noted with morphine in 2 separate studies and once with demerol. The urine flow rose to 2.1 cc. and 2.09 cc. per minute and under the influence of the drugs fell to 0.24 cc. and 0.95 cc. per minute. In addition, renal plasma flow as determined by the para-amino-hippuric acid clearance test[22] was studied during 1 series of observations. This renal plasma flow showed no variation from the normal figure

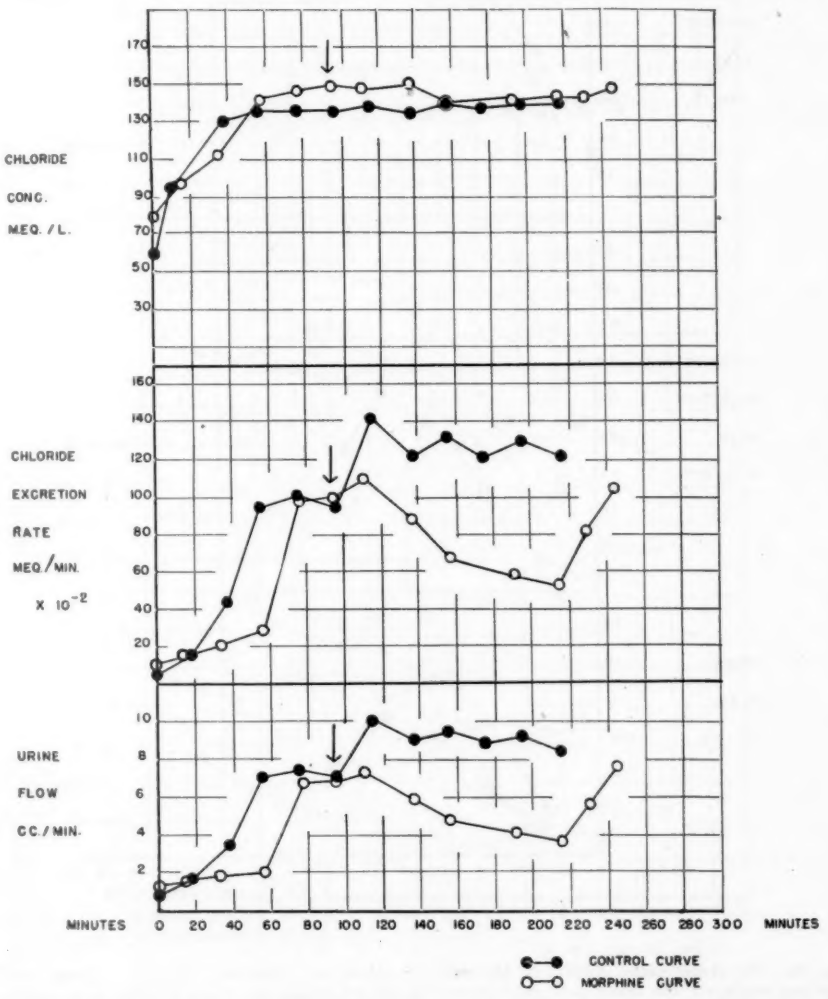

FIG. 1.—*The Antidiuretic Action of Morphine on Mercurial Diuresis.* (C. P.) Closed circles represent the control curve with mercupurin alone. Open circles represent the curve under the influence of morphine. The axis of abscissæ represents the time in minutes after the mercupurin injection, and the axis of ordinates the urine flow in cc. per minute, chloride excretion rate in milliequivalents per minute, and chloride concentration in milliequivalents per liter. The arrow marks the time of morphine injection on the open circle curve. The antidiuretic effect begins 40 minutes after morphine is injected and is maximal at 120 minutes after morphine.

either during the control diuretic period with mercupurin alone, or following mercupurin and demerol. During the antidiuretic effect of morphine in this patient there was a definite fall in urine chloride concentration (Fig. 5). This fall was not demonstrated by the first patient whose chloride concentration remained constant during morphine antidiuresis.

A third patient (M. H.) was studied once with morphine. A control curve was also obtained. This patient showed a

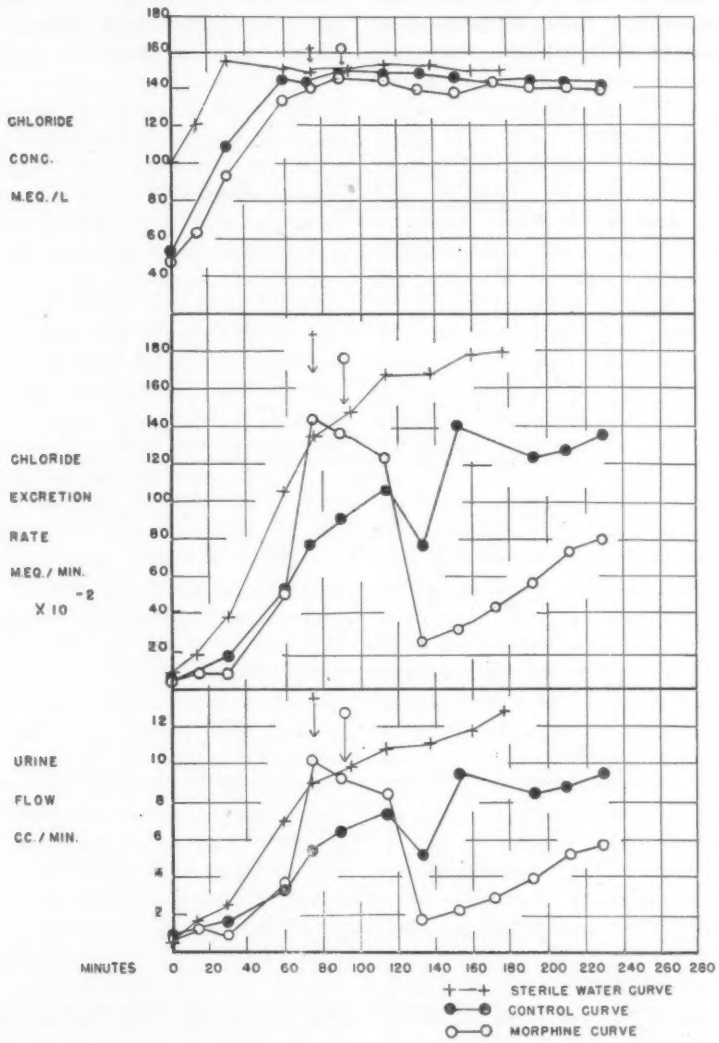

FIG. 2.—*The Antidiuretic Action of Morphine on Mercurial Diuresis.* (C. P.) These 3 diuretic curves are made on the same patient. Closed circles represent the control curve with mercupurin alone. Open circles represent the curve showing the influence of morphine and the cross marks a curve, showing the lack of influence of a sterile water injection on the mercurial diuretic curve. The arrow with the circle above it represents the time of injection of morphine on the open circle curve. The arrow with the cross mark above it represents the time of injection of the sterile water. The axis of abscissæ represents the time in minutes after the mercupurin injection, and the axis of ordinates the urine flow in cc. per minutes, chloride excretion rate in milliequivalents per minute, and chloride concentration in milliequivalents per liter.

somewhat less marked antidiuretic effect with morphine. The urine flow fell from 3.24 cc. to 2.03 cc. per minute. Demerol was not studied in this patient.

The 2 patients who showed an antidiuretic effect on 3 separate occasions demonstrate the consistent nature of this effect and rule out any possible chance phenomenon.

There were 6 cardiac patients who showed no antidiuretic effect with morphine during mercurial diuresis. It should be noted that perhaps a larger dose of morphine would have given an antidiuretic effect in these patients.

Normal Subjects. The first normal subject (F. St.C, Fig. 6) received the second administration of water by mouth: 1000 cc.

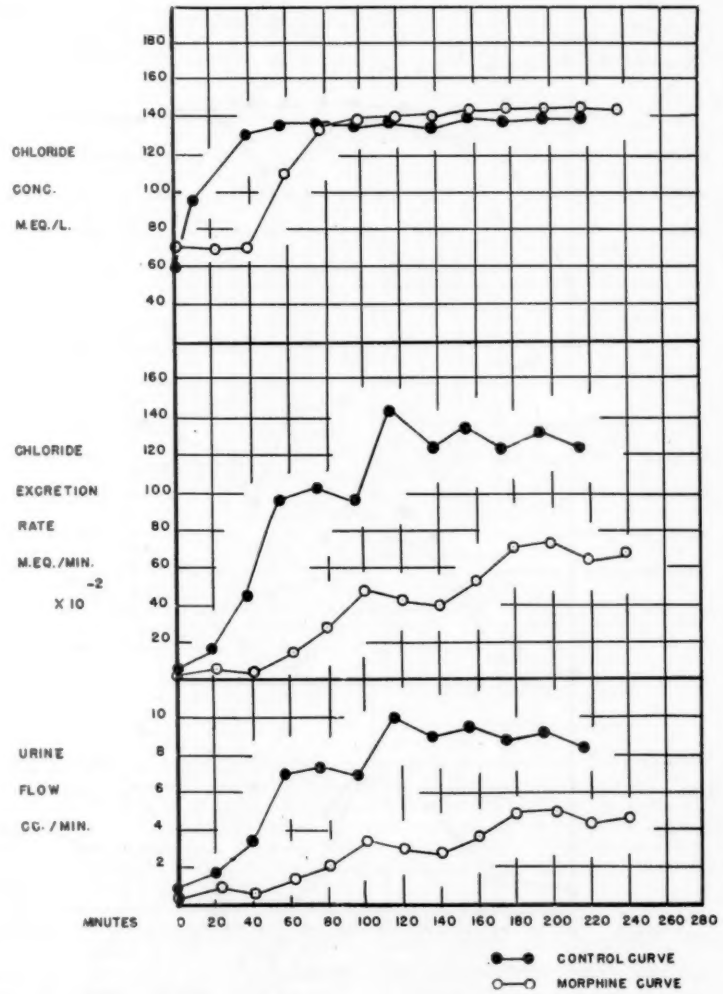

FIG. 3.—*The Antidiuretic Action of Morphine in Mercurial Diuresis.* (C. P.) Morphine sulfate was given 10 minutes before the mercupurin in this study. Note the generally low diuretic curve that followed and which had not risen to the control level at the end of 4 hours. Closed circles represent the curve with mercupurin alone. Open circles mark the curve under the influence of morphine. The axis of abscissæ represents the same in minutes after the mercupurin injection and the axis of ordinates, the urine flow in cc. per minute, chloride excretion rate in milliequivalents per minute, and chloride concentration in milliequivalents per liter.

in 5 minutes. Twenty minutes after the water was given she received 0.01 gm. of morphine intramuscularly. Forty minutes after morphine was given, and 60 minutes after the second ingestion of water, there was a marked drop in the urine flow; from 4.8 cc. to 1.33 cc. per minute. As can be seen in Figure 6, the control diuretic curve made 2 days later on this patient, with only the second ingestion of water (no morphine) shows that at 60 minutes after the second water the urine flow had risen to 8.4 cc. per minute. The antidiuretic effect of morphine in this patient continued for over 2 hours after the morphine was given. At the end of 3 hours the control period yielded 114% of the second water as

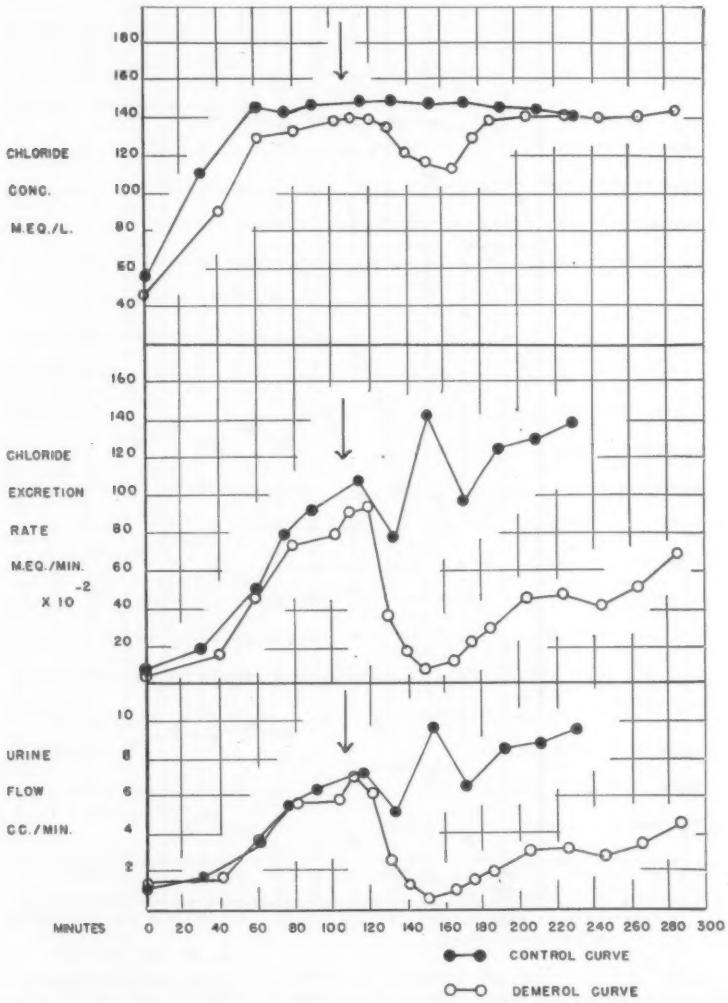

FIG. 4.—*The Antidiuretic Action of Demerol on Mercurial Diuresis.* (C. P.) Seventy-five mg. of demerol were given intramuscularly at the time marked by the arrow. Closed circles represent the control curve with mercupurin alone. Open circles represent the curve under the influence of demerol. The axis of abscissæ represents the time in minutes after the injection of mercupurin and the axis of ordinates, the urine flow in cc. per minute, chloride excretion in milliequivalents per minute, and chloride concentration in milliequivalents per liter. A slight fall in urine chloride concentration was noted during the antidiuretic effect of demerol.

urine returned. This demonstrates the fact that the patient was completely hydrated by the first water given. Under the influence of morphine only 70% of the second water was recovered in the urine. It will also be noted in Figure 6 that the urine chloride concentration rose markedly during the antidiuretic action of morphine. An initial level of 35.8 milliequivalents per liter rose to a maximum of 90 milliequivalents per liter, while control levels started at 13.3 and fell to 1.3 milliequivalents per liter during the control diuretic period. The chloride excretion rate was higher with morphine than the control rate, and the total chloride excretion in 3 hours was greater with morphine, being 19 milliequivalents (1.11 gm.), while the control excretion was 2.8 milliequivalents (0.17 gm.). Therefore, the total urinary chloride excretion increased during the period of morphine action.

Morphine, however, in some subjects, may hinder the absorption of water from the alimentary canal and the decreased excretion reflects this fact rather than true antidiuretic action. Thus, while water diuresis usually reaches its peak 40 to 60 minutes after water is taken, suggesting that the greater part of this water has been absorbed by this time, we were

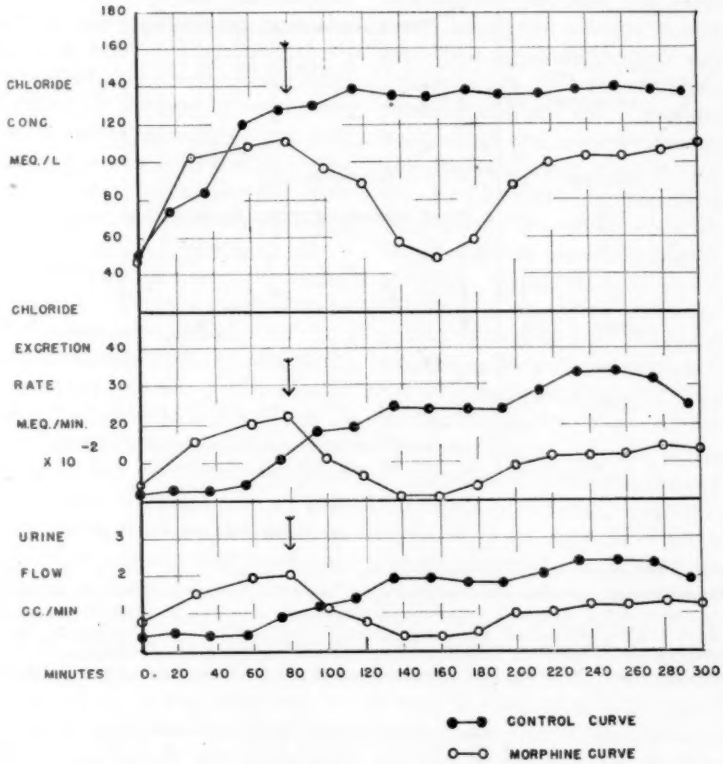

FIG. 5.—*The Antidiuretic Action of Morphine on Mercurial Diuresis.* (S. W.) Ten mg. of morphine sulfate was injected at the time marked by the arrow on the open circle curve. The closed circles represent the control curve with mercupurin alone. The open circles delineate the curve under the influence of morphine. The axis of abscissæ represents the time in minutes after the injection of mercupurin, and the axis of ordinates the urine flow in cc. per minute, chloride excretion rate in milliequivalents per minute and chloride concentration in milliequivalents per liter. Note the fall in urine chloride concentration during antidiuresis.

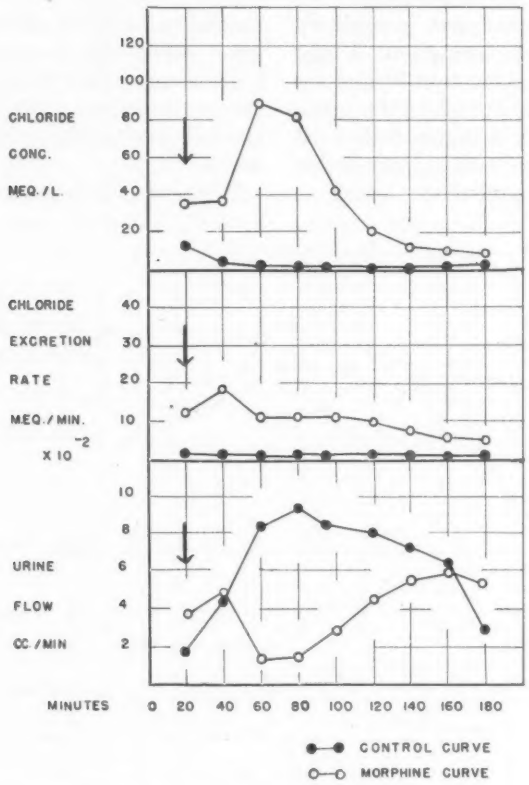

FIG. 6.—*The Antidiuretic Action of Morphine on Water Diuresis.* (F. St.C.) The arrow marks the time of the morphine injection. Closed circles represent the control curve of simple water diuresis. Open circles represent the influence of morphine on the water diuresis. The axis of abscissæ represents the time in minutes after the second water was administered, and the axis of ordinates the urine flow in cc. per minute, chloride excretion rate in milliequivalents per minute, and chloride concentration in milliequivalents per liter. Note the rise in urine chloride concentration during the antidiuretic action of morphine.

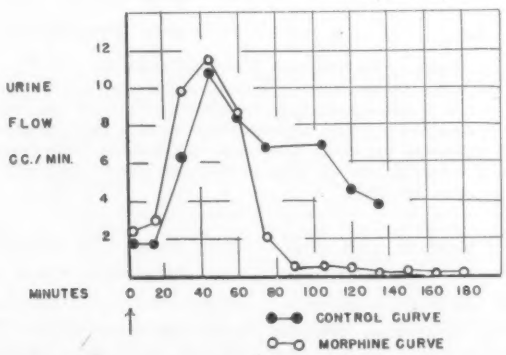

FIG. 7.—*The Antidiuretic Action of Morphine on Infusion Diuresis.* (M. McC.) The arrow marks the time of the morphine injection. Closed circles represent the control curve with simple infusion diuresis. Open circles mark the curve showing the influence of morphine on the infusion diuresis. The axis of abscissæ represents the time in minutes after the infusion was started. The axis of ordinates marks the urine flow in cc. per minute.

able to recover from the stomach of 1 normal subject 525 cc. of a 1000 cc. dose of water during this period, which was also 20 minutes after 0.015 gm. of morphine sulfate was administered intramuscularly.

In our patient, F. St.C. (Fig. 6), however, the urine chloride concentration rose during the antidiuresis and this indicated that the fall in water output was not due simply to diminished absorption from the gastro-intestinal tract. Therefore, these observations probably demonstrate true inhibition of water diuresis by morphine.

In using the intravenous route it would not be safe to give a large dose of unmodified distilled water rapidly. For this reason distilled water was given with 5% glucose. Under these conditions, however, we are no longer dealing with a simple water diuresis. Three normal subjects were studied using this method.

The second normal subject, M. McC. (Fig. 7), received the second water as 1000 cc. of 5% glucose in water given in 40 minutes. Since the factor of absorption plays no rôle in the fluid administration here, the morphine sulfate, 0.01 gm. was given at the same time as the infusion was started. Sixty minutes after morphine was given, urine flow was 8.6 cc. per minute (Fig. 7). Seventy-five minutes after morphine (this is also 75 minutes after the infusion was started) the urine had fallen to 2.2 cc. per minute and at 90 minutes after morphine the urine flow fell to 0.6 cc. per minute and remained under 0.5 cc. per minute until the study was completed 3 hours after morphine was given. A control curve obtained 1 week before remained between 6.7 and 3.8 cc. per minute from 75 to 135 minutes (2½ hours) after the infusion was started. In this patient no chloride determinations were made.

In a third subject morphine was given 1 hour before the infusion was started in order to learn if the diuresis could be suppressed before it had been initiated. This patient showed no antidiuretic effect and excreted 102% of the 1000 cc. in 2 hours after the infusion was started.

A fourth subject received the morphine 70 minutes and 15 minutes before the infusion on 2 separate occasions. A control curve was also obtained. Morphine had no apparent effect on these 2 occasions.

The antidiuretic effect, therefore, was noted in 2 of the 4 normal subjects studied. Again it should also be noted that the dose of morphine used here was relatively small and conceivably a larger one might have made the antidiuretic effect apparent.

Discussion. That morphine inhibits diuresis due to water has been firmly established in several animal species. The demonstration that it has a similar action in humans presents great technical difficulties. To initiate diuresis, one may administer the water by mouth or intravenously, as was done in this study. In the first place, when water is given by mouth, it may be possible to demonstrate a decrease in water excretion during the period of morphine action. This is noted in Fee's study[16] and in our own patient (F. St.C., Fig. 6). In our patient the rise in chloride excretion is a further check on the morphine action. It must be remembered, however, that all of the water may not be absorbed, as demonstrated in the patient studied by means of stomach tube, who still had over half the initial water dose in the stomach 40 to 60 minutes after it was given. Decreased percentual return of the water thus cannot be used as an evidence of morphine action, unless a check of the amount of water leaving the stomach is made. In the second group of normals studied, true simple water diuresis was not established, as glucose in itself influences diuresis.

However, from the results obtained it is apparent that morphine and demerol exert an antidiuretic effect on mercurial diuresis in some patients with congestive heart failure. Morphine was also shown to inhibit a simple water diuresis and an infusion diuresis in some normal persons. The antidiuretic effect was noted as early as 40 minutes and as late as 75 minutes after morphine was given and persisted for a period of 2½ to 4 hours. The anti-

diuretic effect of demerol was noted somewhat sooner, 24 minutes after the injection, and persisted 2 to 3 hours.

It was of clinical interest that 1 cardiac patient (C. P., Figs. 1 to 4) reported to us after the study that before she came under our care her physician had sedated her with frequent oral tablets of morphine and that injections of mercupurin did not result in diuresis. Another cardiac patient (S. W., Fig. 5) volunteered the information that diuresis was delayed for 12 hours when she had been given morphine with the mercupurin.

Mechanism of the Antidiuretic Action. Renal ischemia might be suspected, as it is known that morphine causes liberation of epinephrine[14] in dogs and epinephrine can cause diminished renal blood flow. De Bodo,[12] however, disproved this mechanism by showing that in dogs, with 1 adrenal gland extirpated and the second denervated, morphine caused inhibition of water diuresis to the same extent as in the normal dog. In our patient (S. W.) there was no change in renal plasma flow during the antidiuretic period. This type of renal function study during narcosis, as done by Corcoran and Page[9] and Craig, Visscher and Houck,[10] has been criticized recently by de Bodo[13] because agents in themselves influencing diuresis (such as glucose, saline, etc.) have been included in the infusion material. Fee, in 1928, suggested a mechanism involving the pituitary. De Bodo is of the opinion that morphine inhibits water diuresis by an action upon the hypothalamic-hypophyseal system, probably by increasing the secretion or liberation of the antidiuretic hormone. He and his co-workers,[11,12,13] working on dogs, carried out a careful differentiation of the regions of the pituitary that were necessary for the antidiuretic action of morphine. Their work suggested that a functioning neurohypophysis was necessary for this action. The liberation of this pituitary hormone could be a release phenomenon, or morphine could stimulate the hypothalamic-hypophyseal system either by action on the supra-optic or para-ventricular nuclei or on the pituitrin secreting cells themselves. The intimate details of the mechanism are, of course, not yet known. Pickford,[29] in a recent review, is of the opinion that the pituitary mechanism need not represent the sole explanation of the antidiuretic action.

Chloride Excretion. De Bodo concludes that morphine achieves its antidiuretic action by means of the antidiuretic hormone and bases this conclusion firstly on the fact that morphine and the hormone have analogous actions on water diuresis. In enlarging on this analogous action, he further points out that the neurohypophyseal antidiuretic hormone brings about a percentual and absolute increase in urinary chloride excretion. The chloride increase was also noted in dogs during morphine antidiuresis. A third point of similarity of action between the antidiuretic hormone and morphine is that neither reagent inhibits saline diuresis as a rule, but if any suppression occurs it is of a lesser degree than in water diuresis. In summary, therefore, morphine in animals appears to have the same effect on water diuresis, chloride excretion, and saline diuresis as the antidiuretic hormone, all points in favor of the action of morphine being due to the liberation of the hormone. Saline diuresis in dogs is not affected by morphine. This does not appear to be the case with phenobarbital which, in some animals, does inhibit saline diuresis.

In 2 of the 4 studies of water diuresis in man, morphine exerted an antidiuretic effect. Morphine also brought about an increase in total urinary chloride excretion in 1 patient so studied; 2.79 milliequivalents (0.166 gm.) during the control 3 hours, and 19 milliequivalents (1.11 gm.) during the morphine effect (F. St.C, Fig. 6). In the studies with mercurial diuresis, however, entirely different results with chloride excretion were obtained.

In man, the normal concentration of urinary chloride is about 102.5 milli-

equivalents per liter or 6 gm. per liter. The 24 hour chloride excretion of a normal adult studied for 16 consecutive days fluctuated between 60 and 130 milliequivalents or 3.5 and 7.7 gm.[20] In a 4 hour period, therefore, the estimated total chloride excreted would be 10 to 22 milliequivalents or 0.58 to 1.28 gm. of chloride. In man, diuresis following mercurials is known to be accompanied by a marked increase in chloride excretion. In our patients, the concentration of urinary chloride rose markedly from a control range of 50 to 70 milliequivalents (2.92 to 4.08 gm.) per liter before mercupurin was given, to a range of 100 to 150 milliequivalents (5.8 to 8.77 gm.) per liter following mercupurin. (Figs. 1 to 5.) This elevated concentration was reached early, usually within the 1st hour after mercupurin was injected and occasionally before diuresis was noted, and was maintained at a constant level throughout the next 3 to 4 hours. In 2 of the 3 patients showing an antidiuretic effect the urine chloride concentrations remained approximately the same during antidiuretic as during the control periods. In 1 patient following morphine (S. W.) the chloride concentration fell to the control levels obtained before mercupurin was given and only rose again at the end of the antidiuretic effect. Morphine, in this case, therefore, caused a decrease in the chloride concentration in the urine. In all 3 patients, however, the total chloride excreted in the urine during the antidiuretic period as compared with each control period was considerably reduced. Results are outlined in Table 1.

The normal chloride excretion during any 4 hour period of the day has been estimated as 10 to 22 milliequivalents (0.58 to 1.28 gm.). Comparison of this figure with the total chloride excreted the 4 hours following the injection of mercupurin (see Table 1) emphasized the large mobilization of chloride affected by mercupurin. This mobilization of chloride, as measured in our observations by the total chloride excretion during the period of study, was considerably reduced during morphine and demerol antidiuresis.

TABLE 1.—THE EFFECT OF MORPHINE AND DEMEROL ON CHLORIDE EXCRETION DURING MERCURIAL DIURESIS IN MAN

Patient	Control diuretic curves	During morphine antidiuresis	During demerol antidiuresis
Total Chloride Excreted in the Urine During 4 Hours Following Mercupurin			
C. P.	255.0 m.eq. (14.9 gm.)	171.2 m.eq. (10.0 gm.)	102.9 m.eq. (6.0 gm.)
	219.0 m.eq. (12.8 gm.)	159.7 m.eq. (9.34 gm.)	
	314.2 m.eq. (18.35 gm.)	104.5 m.eq. (6.1 gm.)	
M. H.	155.5 m.eq. (9.08 gm.)	74.5 m.eq. (4.35 gm.)	
Total Chloride Excreted in the Urine During 5 Hours Following Mercupurin			
S. W.	63.3 m.eq. (3.7 gm.)	18.4 m.eq. (1.08 gm.)	
	57.6 m.eq. (3.36 gm.)		

In our observations in man, morphine caused an antidiuretic effect and a slight increase in chloride excretion during the studies on water diuresis. This agrees favorably with De Bodo's animal studies. It is apparent, however, that morphine had a similar antidiuretic effect on mercurial diuresis but a depressant effect on chloride excretion during this diuresis. If mercurial diuresis can be considered at least in part a saline diuresis, then in man it can be said that morphine does depress or inhibit saline diuresis.

Conclusions. 1. The influence of morphine on mercurial diuresis was studied in

9 patients with congestive heart failure. In 3 morphine produced an antidiuretic effect. In 2 patients demerol also showed an antidiuretic effect.

2. The total chloride excretion during mercurial diuresis was reduced by morphine and demerol.

3. Under certain circumstances it is possible to demonstrate the inhibition of water diuresis in man by morphine. The total chloride excretion during water diuresis was increased by morphine.

4. It is suggested that the antidiuretic effect of morphine and demerol, while probably not operative in all patients, cardiacs or otherwise, is of sufficient importance in some cases of cardiac decompensation to account for certain poor results with mercurial diuretics.

The authors would like to express their thanks to Dr. Arthur C. DeGraff for his suggestions and help which he has given us in this work.

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FAILURE OF STREPTOMYCIN IN THE TREATMENT OF LEPROSY

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The ability of streptomycin to inhibit the growth of *Mycobacterium tuberculosis, in vivo* and *vitro*, has been demonstrated by numerous investigators.[1–5]

Because *Mycobacterium lepræ* resembles *M. tuberculosis* in its morphology, staining qualities and in its tendency to produce chronic granulomata, it seemed reasonable to attempt the treatment of leprosy with streptomycin, after no report of this use of streptomycin could be found in the literature.

Case History. P. O., a Filipino steward's mate, was admitted to the hospital on Sept. 14, 1946, complaining of areas of dark and of depigmented skin over legs and trunk. He stated that while working in Guam in July 1946, he had stumbled into mud up to his knees while carrying a heavy box, and shortly after noted an itching and scaling of both legs. The lesions spread downward over the feet and upward to his knees. In August 1946, he noted that patches of skin over his chest were losing their normal pigmentation. The past medical history was not remarkable, save that he had been bitten by a dog in 1944. He enlisted in the Navy as a steward's mate in the Philippine Islands on May 29, 1946, at which time no physical abnormalities of the skin were noted in his health record, and the patient stated that none existed.

A family history failed to show any instances of leprosy in the immediate or collateral relatives, nor did he remember any contact with any known leper.

The physical examination, at the time of admission, was normal, save for depigmented macules scattered over the trunk, back, arms, buttocks and thighs; infiltrated flat brownish-red plaques over the lateral borders of the feet, and the posterior aspects of the heels; and scaling ichthyotic pigmented plaques over the shins. There was a healed ulcer, 3 cm. in diameter, on the medial aspect of the left leg, 10 cm. above the internal malleolus. There was bilateral inguinal adenopathy. On the first examination there was no evidence of weakness, or wasting of any of the skeletal muscles. No thickening of the peripheral nerves could be found, and there were no areas of anesthesia.

The following investigations were made: Urine: sp. gr. 1.011; reaction, acid; albumin and sugar, negative. Microscopic examination: no abnormalities. A complete blood count was within the limits of normal; RBC, 4,900,000; hemoglobin, 14 gm. (97%); WBC, 8900 (neutrophils, band forms, 2; segmented, 56; lymphocytes, 35; eosinophils, 7). The blood Kahn test was negative. The blood sedimentation rate was 5 mm. in 1 hour.

A roentgenogram of the chest showed moderate prominent bronchovascular markings along the right border of the heart. There was no evidence of active disease of the parenchyma, although the right apex was slightly more dense than the left, which was interpreted as being due to thickening of the pleura. The transverse diameter of the heart was within the limits of normal, and the great vessels were not unusual.

The clinical evidence suggested a diagnosis of leprosy. Scrapings of the nasal mucous membrane and stains of serum obtained from nodular lesions failed to show acid fast organisms. A tuberculin test, on Sept. 23, 1946, using purified protein derivative, in the second dilution was positive in 48 hours. On September 21 a similar test with purified protein derivative in the first dilution, was negative in 48 hours. On September 27, it was demonstrated that the depigmented macular areas on the trunk were hypesthetic. Repeated attempts were made to obtain *M. lepræ* from the nasal mucous membranes and from the nodular and depigmented macular lesions of the trunk and extremities without success. A biopsy was obtained from a nodular lesion on the left leg, and on November 1 acid fast organisms were demonstrated in sections obtained from this material. Because of the clinical evidence of leprosy and the finding of acid fast organ-

* This work was done while serving as Chief of Medicine, United States Naval Hospital, Treasure Island, San Francisco.

isms in the skin, no attempt was made to cultivate the organism on artificial media or in animals. Photographs were taken of the lesions of the skin (Fig. 1). A reëxamination of the patient, at this time, revealed areas of anesthesia over the right foot, and over the depigmented macular areas of the trunk and extremities. There were depigmented macules scattered over the back, arms, buttocks and thighs, and infiltrated brownish-red plaques over the lateral borders of the foot, posterior aspect of the heel, and an occasional macule on the buttocks. There were ichthyotic plaques over the shins and hypesthesia approaching anesthesia of the skin, of the lesions, on the buttocks and thighs, and of the lateral and posterior aspects of the feet. A slight cyanosis of the hands, and loss of tone with slight flattening of the hypothenar eminence of the right hand, and atrophy of the muscles of the second interosseous space of the right hand was noted. There was slight drooping of the lower right eyelid. No thickening of the peripheral nerves could be demonstrated. The sedimentation rate was 15 mm. in 1 hour. The complete blood count was similar to the one taken on admission, and a second blood Kahn test was negative. On November 1, streptomycin therapy was started. The patient was given an intramuscular injection of 4 gm. of streptomycin as an initial dose; and 2 gm. were given intramuscularly every 2 hours for 72 hours, followed thereafter by 0.5 gm. every 4 hours, throughout the course of treatment. On November 2 he com-

FIG. 1.—Photograph illustrating ichthyotic plaques over the shins before treatment.

plained of severe vertigo when attempting to stand erect or walk. On November 5 the sedimentation rate was 11 mm. in 1 hour. On November 7 the vertigo had subsided and it was noted that the scaling lesions over the legs were disappearing and the epidermis appeared nearer normal. The areas of hyperpigmentation and depigmentation were unaffected. At this time, the sedimentation rate was 2 mm. in 1 hour. On November 29 the patient complained of pain in both external ears, and areas of hyperpigmentation were noted along the helices. On December 7 he complained of some dizziness, as well as pain over the nodular lesions on the skin, which appeared to be darker in color. On December 18 he complained of pain in the lobule of the right

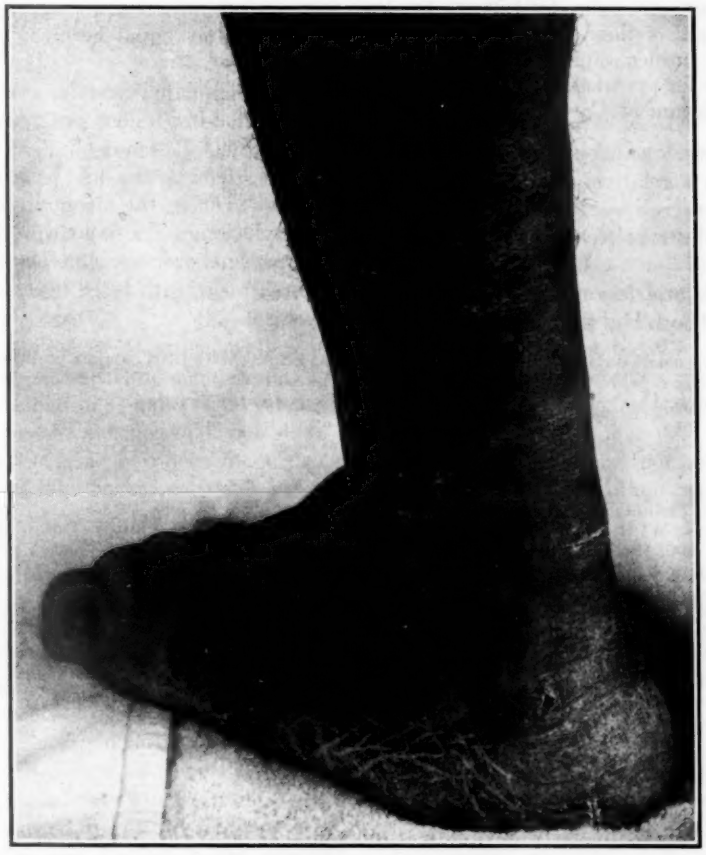

FIG. 2.—Left foot showing hyperpigmented nodular lesions and the remains of the ichthyotic plagues following therapy with streptomycin.

patient noted some pain in the areas of hyperpigmentation, about the bases of the toes, and the dorsal surfaces of both feet. There was no change in the neurologic examination. On November 19, photographs were taken of the lesions of the skin (Fig. 2). It was noted that the hyperpigmented areas over the feet had become anesthetic and had increased in size. The ichthyotic lesions continued to improve. On November 20 ear, where a nodule had formed. He stated that the pain was worse when the room was warm. On December 10 the patient was seen by Dr. N. E. Wayson, Medical Director of the U. S. Public Health Service, who confirmed the diagnosis of leprosy. On December 26 streptomycin was discontiuued. The patient had received 175 gm. of streptomycin over a period of 55 days. On December 31 all evidence of vertigo had subsided

and a biopsy was obtained from a lesion similar to, and adjacent to the lesion from which the original biopsy was taken. The tissue obtained at this time showed acid fast organisms morphologically resembling *M. lepræ* in approximately the same number and distribution, as those seen in the original biopsy. By Jan. 13, 1947, the ichthyotic areas over both shins had returned, and resembled the lesions prior to the beginning of treatment. On February 1 it was decided that the trial of therapy with streptomycin had been unsuccessful, and arrangements were made for the transfer of this patient to the leprosarium at Carville, Louisiana.

Summary. 1. A patient suffering from leprosy in a relatively advanced and apparently progressive stage was treated with 175 gm. of streptomycin over a period of 55 days.

2. Ichthyotic lesions over the anterior surfaces of both legs showed improvement, but returned when streptomycin was discontinued.

3. The sedimentation rate dropped from 15 mm. in 1 hour to 2 mm. in 1 hour, during the course of therapy, but returned to 11 mm. in 1 hour, 1 month after treatment was discontinued.

4. Biopsies obtained before and after treatment showed acid fast organisms morphologically resembling *M. lepræ* in approximately equal numbers and distribution.

5. Depigmented macular and hyperpigmented nodular lesions progressed in spite of therapy.

6. Vertigo was the only toxic manifestation noted from the streptomycin.

Conclusion. The course of leprosy in this patient was not significantly altered by treatment with large doses of streptomycin.

It is my pleasant duty to thank George G. Herman, Capt. (MC) USN, for his permission to carry out these studies; N. E. Wayson, Col., U. S. Public Health Service, for his kind suggestions and help; and D. Oborne, Lt. Comdr. H(W), for the histologic sections used in this study.

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PSITTACOSIS

REPORT OF AN OUTBREAK

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Psittacosis is a rare disease in Massachusetts. From August 1938, the date it was declared a reportable disease, to April 1946, when the outbreak to be described in this report occurred, not a single case had been recorded. However, from December 1929 to March 1930, a series of 15 cases came to the attention of the Massachusetts Department of Public Health. Investigation revealed that each patient had been exposed to infected parrots in one or another of 2 pet shops. Three sporadic cases had been noted prior to 1938, 2 in 1932 and 1 in 1934.

It is difficult to obtain a reliable estimate of the true incidence of psittacosis. Unless a history of exposure to psittacine or other birds which may transmit the infection is elicited, cases of ornithosis may not be recognized. Routine testing of acute and convalescent phase sera obtained from cases of virus pneumonia for the presence of psittacosis complement fixing antibodies would result in the recognition of additional cases. Similarly, ornithosis caused by psittacosis-like agents of non-psittacine derivation are equally difficult to detect.

Clinically ornithosis resembles atypical pneumonia to such an extent that confusion in the diagnosis of these diseases is quite understandable. At times the pulmonary involvement that occurs in both conditions is demonstrable only by Roentgen ray. The white blood count and the clinical course may be similar and of limited value in the differential diagnosis. For these reasons, without special laboratory aids, it is possible that cases of ornithosis may be misdiagnosed as virus pneumonia.

It is proposed in this discussion to present the clinical, laboratory and epidemiologic aspects of a small outbreak of psittacosis which occurred in Massachusetts during April and May 1946. Serologic tests revealed a possible third case in a person who had been exposed to birds in 1 of the aviaries concerned in the outbreak. Blood specimens from 2 other exposed persons who had not been ill were positive in a low dilution, suggesting inapparent infection.

Case Reports. Case 1. Mrs. C. E., a 44 year old, white, married housewife, was referred on April 24, 1946, to the Faulkner Hospital, Boston, Mass., under the care of Dr. Channing Frothingham, with a complaint of recurrent daily fever that had risen as high as 103° F. She had been quite well until about 4 days before admission when she noticed the onset of slight fever, general malaise and headache. Three days later the elevated temperature and headache were still present and the patient had developed generalized muscle pain. She gave no history of cough, dyspnea or recent upper respiratory infection. Chest pain and other symptoms of pneumonia were lacking.

Physical Examination. The patient presented the picture of an acute infection with hot, dry skin, anxious expression and marked prostration. The temperature was 103° F., pulse rate 100 and respiratory rate 22. General physical examination was negative.

Laboratory Studies. A blood culture, upon admission before treatment was instituted,

showed no growth. The white count was 7800 with a normal blood smear. Subsequent white counts on April 28 and 30 were 5100 and 7100 respectively, with normal blood smears. All urine and stool examinations were negative. Roentgen ray of the chest was reported by Dr. Harvey Morrison as follows: "The findings are those of 3 areas of pneumonic infiltration, 1 in the right upper lobe, 1 in the left upper lobe and the third in the left lower lobe. The appearance is more that of an atypical pneumonia,

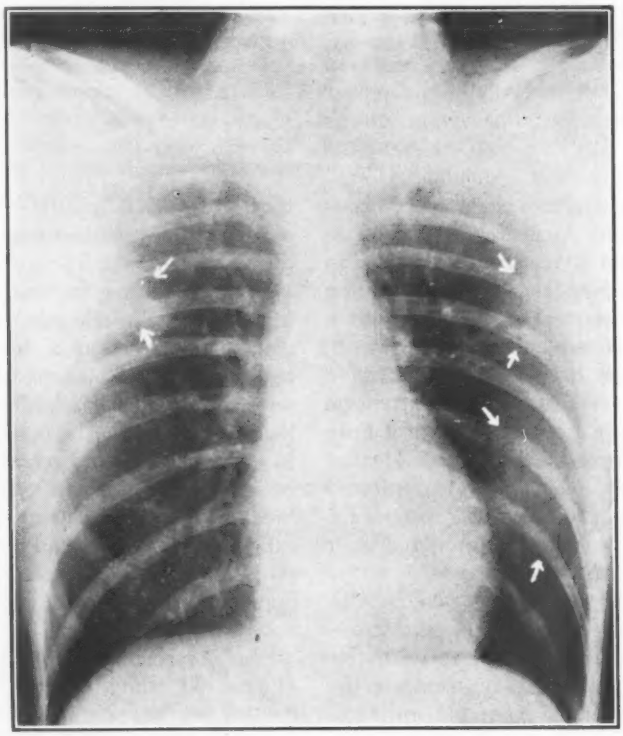

FIG. 1.—Chest Roentgen ray of Mrs. C. E. (Case 1) showing 3 areas of pneumonic infiltration as indicated by the arrows.

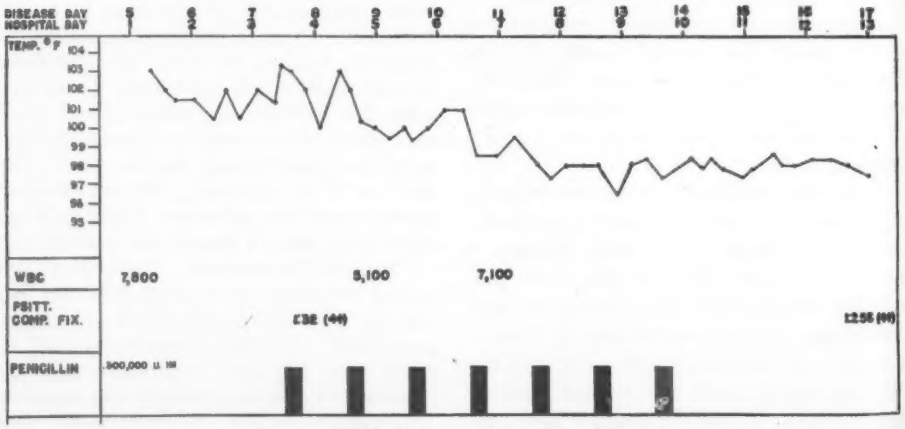

FIG. 2.—Clinical chart of Mrs. C. E. (Case 1).

although such unusual types of pneumonia as eosinophilic pneumonia cannot be excluded. There is nothing inconsistent with psittacosis." (Fig. 1.)

Blood samples for psittacosis complement fixation tests, sent to Dr. K. F. Meyer of California, were reported as follows:

Date sample taken	*Titer*
April 27	1:32 4+, 1:64 2+
May 6	1:256 4+, 1:512 2+
June 1	1:512 4+

Clinical Course. Treatment with penicillin was instituted on the 3rd hospital day; 300,000 units in beeswax and peanut oil were administered daily for 7 days. The temperature dropped slowly by lysis and within 4 days had returned to normal. (Fig. 2.)

There was no cough throughout the entire illness and no physical findings suggestive of pneumonia. On the 3rd day of hospitalization the patient complained of some pain in the left chest upon deep inspiration. The heart rate seemed to be relatively slow despite the elevated temperature. The patient's complaints of headache and muscle pain gradually disappeared and the temperature fell. Appetite and general well-being returned slowly and she was discharged on the 12th day.

Case 2. Mr. A. M., a 31 year old, white male, entered the Cambridge Hospital, Cambridge, Mass., on April 30, 1946, with a history of severe headache and chills for 5 days. Four days prior to admission he was unable to continue at work and upon arrival at his home his temperature was found to be 103° F. The headache persisted without relief. There was moderate anorexia, but no other unusual symptoms. On the 7th day of his illness, the patient had diaphoresis associated with a remission of the fever. There had been a very slight, infrequent cough without chest pain, sputum, dyspnea or orthopnea.

Physical Examination. On admission the temperature was 102.6° F., pulse 100, respiration 28. Patient was a very well-developed and well-nourished male in no acute distress. The physical examination was essentially negative.

Laboratory Studies. Initial blood study: red blood count 4,320,000, hemoglobin 91% (Sahli); white blood count 6500 (neutrophils 68%, lymphocytes 29%, monocytes 3%). Urinalysis and the Hinton test were negative. On April 30 blood culture showed *S. albus* (probably a contaminant). Roentgen ray of chest on May 1 was read as follows: "There is some bronchitis at the right base. The lungs are otherwise normal. The heart is normal." A repeat examination on May 8 was read: "Reëxamination shows a definite improvement in the infectious process involving the right lower lobe."

Blood samples for psittacosis complement fixation tests were sent to Dr. K. F. Meyer and were reported as follows:

Date sample taken	*Titer*
April 30	1:2 4+
May 8	1:32 4+
June 11	1:64 4+, 1:128 3+, 1:256 2+

Clinical Course. The temperature remained elevated for 3 days. Repeated physical examination of the chest revealed no abnormal findings. The cough remained unproductive and gradually disappeared during the hospital stay. Recovery was uneventful and patient was discharged on May 10, 1946.

Epidemiology. The epidemiologic investigation revealed that both patients had been in contact with sick parakeets, several of which had died. A. E., husband of C. E. (Case 1), maintained an aviary (Aviary A) at his home. Both the husband and the wife cared for the birds. A. M. (Case 2) was a neighbor who had been exposed to the birds in this aviary on several occasions during the month prior to the onset of his illness. A sample of blood taken from A. E. showed a positive complement fixation test for psittacosis in a serum dilution of 1:64 4+, 1:128 3+. He had not been ill.

Several weeks prior to the occurrence of illness among birds of his aviary, A. E. had purchased several parakeets from the owner of another aviary (Aviary B). There had been several deaths among the parakeets in Aviary B. Recent additions of psittacine birds to both of these flocks had been made from aviaries outside of Massachusetts.

Investigation among persons exposed to Aviary B revealed that 1 individual, a

Dr. M., had developed a febrile illness associated with pulmonary signs and symptoms on Dec. 13, 1945. He had been hospitalized with a diagnosis of lobar pneumonia. A chest Roentgen ray taken during the hospital stay showed a shadow at the left base. The white blood count was 9000, with 68% polymorphonuclears. Blood specimens taken from this patient and Mrs. A, the owner of Aviary B, were sent to Dr. K. F. Meyer and were reported as follows: specimen from Dr. M. was positive in dilution of 1:16 4+, 1:32 2+; that of Mrs. A., 1:8 4+ and 1:16 2+. In the absence of the acute phase complement fixation test for psittacosis on Dr. M., the evidence that his illness was caused by the virus of psittacosis is only presumptive.

The National Institute of Health isolated the virus of psittacosis from a sick parakeet in Aviary A. The remainder of the birds in this aviary were destroyed. The birds in Aviary B were placed under quarantine for several months. No birds became ill during the period of observation. Two ricebirds, a species that are highly susceptible to the virus of psittacosis, were admitted to this aviary but did not develop psittacosis.

Comment. The paucity of reported cases of psittacosis may indicate either low incidence or failure of recognition. A study by Meiklejohn *et al.*[8] showed that when 250 specimens of sputum and lung tissue from patients with primary atypical pneumonia were inoculated into mice and cotton rats, only 10 positive isolations of psittacosis-like viruses were recorded. By use of the complement fixation test, 4 or 5 additional cases of psittacosis-like infection were found from which no virus was isolated. These findings suggest that psittacosis-like virus accounted for only a small portion of the total cases of primary atypical pneumonia.

Favour[2] in 1943 reported 3 cases of ornithosis in Massachusetts. The presumptive source of infection was canaries in 1 case and pigeons in another. No history of contact with birds could be established in the third. In 1946 an additional case of ornithosis in a bird fancier who had been exposed to pigeons was reported to the Massachusetts Department of Public Health.[1]

It has been demonstrated that psittacosis is related antigenically to a virus recovered by Eaton, Beck and Pearson from the lungs of 2 fatal cases of atypical pneumonia.[1] Harrop, Rake and Shaffer showed that laboratory personnel, working with lymphogranuloma virus, developed atypical pneumonia.[4] Cross-reactions in the sera of these patients were found between the virus of psittacosis, the virus of lymphogranuloma venereum, the Eaton virus and Francis and Magill's meningopneumonitis virus.

Because of these cross-reactions, virus isolation should be attempted wherever possible in order to establish a definite etiologic diagnosis. Unfortunately, the present lack of laboratory facilities makes it difficult to attempt virus isolations from large numbers of patients with signs and symptoms suggestive of ornithosis. However, a careful history as to contact with birds, plus the use of acute and convalescent phase complement fixation tests in properly selected cases, is of considerable value in the recognition of ornithosis.

Human infections have been traced to canaries as well as psittacine birds.[9] Chickens, doves and many types of finches are also known to be infected.[10] Since 1941, when it was discovered that pigeons may transmit ornithosis,[11] an increasing amount of attention has been devoted to this species as possible vectors. Studies of pigeons from flocks or lofts in California, New York and South Carolina have resulted in successful isolations of psittacosis viruses.[10]

Two routes of transmission of infection from bird to man are possible. The first is *via* the respiratory tract and the second by direct contact with infected birds. Droppings of the parakeet, parrot, pigeon and fulmar, harbor the virus and may be responsible for respiratory invasion of man by dust. When canaries, finches and hens

are the source of infection the contact is usually direct as in handling of these birds.

Many persons, such as bird fanciers, breeders, pet shop owners and veterinarians, develop ornithosis as the result of occupational contact with birds. Apart from these occupational infections, the majority of reports deal with single or multiple cases in the households of people who have recently purchased infected birds. Children are less susceptible to infection than adults. It has been noted that children frequently escape infection following exposure to parakeets that have infected their parents or other relatives.[10] In rare instances human to human transmission has occurred.[6,7]

The control of psittacosis presents many difficulties. Although the importation of tropical psittacine birds may be regulated by quarantine measures, it is difficult to detect inapparent infections and parrots may be carriers of the virus for many months. Prolonged periods of isolation are essential.

Aviaries yielding infected birds are quarantined and necessary steps are then taken to destroy the entire flock and disinfect the premises. Because of the frequent interchange of birds by dealers and bird fanciers, it is often difficult to trace chains of infection from one aviary to another.

Latent infection in some aviaries may suddenly become activated when birds are subjected to overcrowding, insanitary conditions, changes in diet or unusual activities such as a change in locality.[10] Studies by the California Department of Public Health revealed that the virus was present in many aviaries, the infection being either latent or active.

It will be noted that Mrs. C. E. (Case 1) was treated with penicillin, 300,000 Oxford units having been given each day for 7 days. Heilman and Herrell[5] showed the protective effect of penicillin on mice experimentally infected with a strain of psittacosis virus. Turgasen[12] and Flippin *et al.*[3] reported on the treatment of human cases of ornithosis with penicillin. However, no definite conclusions concerning the efficacy of penicillin should be drawn from these isolated cases. It is apparent that the second patient (Case 2) made an uneventful recovery without the use of penicillin.

Summary. An outbreak of psittacosis in Massachusetts is described. Positive complement fixation tests for psittacosis were obtained from 2 patients, 2 contacts without evidence of clinical disease and 1 additional contact with an illness suggestive of psittacosis. The virus of psittacosis was isolated from a sick parakeet in 1 of the aviaries concerned in the outbreak. The 2 patients involved in the outbreak recovered. Penicillin was administered to 1 while the other was treated symptomatically.

The apparent rarity of psittacosis as suggested by the paucity of reported cases indicates either low incidence or failure of recognition. Careful histories as to contact with psittacine or other birds capable of transmitting ornithosis, plus the use of the complement fixation test, will aid in the detection of cases.

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EXPERIENCES WITH ANTIRETICULAR CYTOTOXIC SERUM (ACS) IN ARTHRITIS*

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In 1942 the representatives of the Academy of Sciences of the Ukranian Soviet Socialist Republic passed a resolution that the therapeutic effect of the Bogomolets antireticular cytotoxic serum (ACS) was clearly established in the treatment of "rheumatism."[3] Sokolinski[7] considered rheumatic disease an allergic process involving active mesenchymal tissue and that ACS was indicated to restore the normal reactivity of connective tissue cells. He treated patients with "acute rheumatism" with ACS and reported that in most of the cases all rheumatic manifestations in the joints subsided within the 1st week. It was further reported by Strazhesko[8] that "In the case of rheumatism, the second period is hyperergic and a single injection of the serum in a stimulating dose stops the disease." Because of these very favorable statements regarding ACS in the treatment of rheumatism it was considered important that the further definition of its value be obtained.

Preparation of Serum. The antireticular cytotoxic serum† was prepared in rabbits according to the method of Marchuk.[4] It had a cytotoxin titer, determined by complement fixation of at least 1:100 and a hemolytic titer below 1:16. Dry lyophilized serum was dissolved in physiologic saline shortly before its use. No serum was injected after 10 days standing in the liquid state, or if the solution became turbid. Frequent complement fixation tests were made to insure potency of the serum.

Experimental Observations. This research consists of 2 parts: (1) observations of the effect of ACS as a prophylactic and therapeutic agent against pleuropneumonia induced arthritis in rats and (2) clinical observation of ACS used to treat rheumatic disease especially chronic arthritis.

Laboratory Animal Studies. Arthritis was produced in rats by the injection of pleuropneumonia-like organisms. White rats, each weighing 100 gm., were inoculated intravenously. One group of animals served as untreated controls. Of a second group some rats were injected with normal rabbit serum while the test animals received potent homologous anti-rat ACS especially prepared for this study. In this group all serum injections were made before inoculation with pleuropneumonia-like organisms to test its prophylactic effect. A third group of rats received injections of ACS simultaneously with the inoculation of pleuropneumonia-like organisms and repeated serum injections on the 3rd and 6th day.

In Table 1 the fate of the rats inoculated with pleuropneumonia-like organisms otherwise untreated is shown. Three of the rats developed arthritis, 2 died of

* Read at the Meeting of The New York Rheumatism Association, New York City, Nov. 22, 1946.
† Obtained through the kindness of Dr. Harry Goldblatt of Western Reserve University, Cleveland, Ohio.

pleuropneumonia and 1 remained well during the 3 weeks of observation. The dependability of pleuropneumonia-like organisms to produce arthritis in rats as previously demonstrated by the work of Sabin,[6] Preston *et al.*,[5] Collier[2] and others is thus corroborated.

The results of attempts to prevent the development of arthritis by injecting antireticular cytotoxic serum prior to inoculation with pleuropneumonia-like organisms are shown in Table 2. Doses ranged from 0.00005 to 0.00015 cc. Three injections were given 9, 6 and 2 days before the introduction of the infective material. In the control group receiving normal rabbit serum, 4 of the rats died, 1 developed arthritis and 1 developed pleuropneumonia. In the group receiving potent ACS serum, 3 of the animals died, 2 developed arthritis and 1 remained normal. It appears from this study that with the technique used, *ACS was of no value in preventing arthritis caused by pleuropneumonia-like organisms.*

Table 3 shows the results of serum injection given simultaneously with and after infective pleuropneumonia-like organisms. Of the control group receiving normal rabbit serum, 3 animals developed arthritis, 1 pleuropneumonia and 2 animals died. In the group receiving ACS there was 1 case of arthritis, 4 deaths from pleuropneumonia and 1 rat remained normal. As used *ACS was of no value as a therapeutic agent against the effects of pleuropneumonia infection.*

CLINICAL INVESTIGATIONS. Observations were made on patients manifesting different stages of activity of rheumatoid arthritis and ankylosing spondylitis. At intervals of 3 or 4 days ACS was injected subcutaneously. The doses were originally 0.5, 1 and 1.5 cc.; later these doses were frequently reduced to 0.3, 0.6 and 0.9 or 1 cc. After intervals of 4 to 6 weeks many patients received a second series of injections, some a third series, and several a fourth series of injections similarly. No changes in other treatment previously administered, which often consisted of physical therapy and salicylates, were made except that 5 patients were hospitalized during treatment.

TABLE 1.—RESULTS IN 6 RATS OF INOCULATION OF PLEUROPNEUMONIA-LIKE ORGANISMS

Result	No. animals
Died of pleuropneumonia within 5 days	2
Arthritis of joints of hind legs	3
Well after 3 weeks	1

TABLE 2.—RESULT IN RATS OF ACS NORMAL SERUM INJECTIONS GIVEN 9, 6 AND 2 DAYS PRIOR TO INOCULATION WITH PLEUROPNEUMONIA-LIKE ORGANISMS

Dosage (cc.)	Normal serum	ACS
0.00005 × 3	Died on 7th day	Died on 5th day
0.00005 × 3	Arthritis	Died on 5th day
0.0001 × 3	Died on 5th day	Died on 12th day
0.0001 × 3	Pleuropneumonia	Normal
0.00015 × 3	Died on 12th day	Arthritis
0.00015 × 3	Died on 12th day	Arthritis

TABLE 3.—RESULTS IN RATS OF ACS AND NORMAL SERUM INJECTIONS GIVEN SIMULTANEOUSLY WITH PLEUROPNEUMONIA-LIKE ORGANISMS

Dosage (cc.)	Normal serum	ACS
0.00005 × 3	Died on 7th day*	Normal
0.00005 × 3	Arthritis	Died on 5th day
0.0001 × 3	Arthritis	Died on 5th day*
0.0001 × 3	Died on 5th day*	Arthritis
0.00015 × 3	Arthritis	Died on 5th day*
0.00015 × 3	Pleuropneumonia	Died on 5th day*

* Three injections planned; animal died after the second injection.

Twenty-nine patients were thus treated with ACS; 24 patients received 2 or more series of injections. Fourteen patients were injected in a similar manner with normal rabbit serum having no antireticular cytotoxic activity. In many instances it was not known until after evaluation of results whether the serum used was ACS or normal (control) serum. Some patients treated with ACS were at another time likewise injected with normal rabbit serum, also given as further control.

Changes in symptoms and physical signs were carefully noted before and after each series of injections; many patients were observed for 6 months or longer after treatment. The alleged stimulation of the connective tissue was followed. The dispersion of trypan blue injected intradermally, which Tatarinov[9] considered to be a dependable index of the activity of connective tissue as a physiologic system, was observed 90 times in 30 patients. A rapid spread of the dye was reported to indicate stimulation of the connective tissue. Although potent ACS was used, no consistent increase in the diffusion of intradermal trypan blue dye was noted. Furthermore the diffusion varies considerably depending upon the depth of injection which could not always be controlled. Because of these facts the reliability of this test was doubted, and so it was abandoned.

Other laboratory tests that have been suggested as indices of activity of the connective tissue are changes in (peripheral) blood morphology, especially an increase in monocytes and a decrease in the erythrocyte sedimentation rate. In all patients a total and differential white blood count and erythrocyte sedimentation rate were determined prior to and following each course of injections. In most cases there was no change in blood morphology. Of the 29 patients who received ACS, only 5 showed an increase in monocytes after injection. The increase exceeded 4% in only 1 instance—when a 7% monocytic response was observed 24 hours after injection of ACS during the time this patient exhibited a moderate tissue inflammation at the site of injection accompanied by systemic fever of 100° F. The monocyte count subsequently fell to 1% and with further ACS treatment the number of monocytes remained at 2% of the total leukocyte count. The erythrocyte sedimentation rate gradually rose in 10 of the 29 patients treated; in 2 cases there was no change, in 17 patients there was a gradual fall in sedimentation rate during and after treatment.

Reactions. Inflammatory reactions at the site of injection were common. Forty-six per cent of the ACS treated patients and 36% of the patients treated with normal rabbit serum manifested some degree of local reaction characterized by heat, swelling, tenderness and usually followed by pruritus. Most reactions occurred following the second or third injection. Inflammation usually started within 24 hours and disappeared within 72 hours. In 4 ACS treated patients systemic reactions evidenced by chills, fever, malaise and occasional axillary lymphadenopathy occurred. One patient developed generalized urticaria during the first series of injections. No systemic reactions occurred in the patients injected with normal serum. No relationship was noted between clinical improvement and the severity of reaction.

Clinical Results. Table 4 summarizes the clinical evaluation of 29 patients treated with ACS and 14 patients injected with normal rabbit serum. Three patients who received ACS improved symptomatically. Three showed objective improvement; in 16 there was no change and 7 became worse. Of the control group (injected with normal rabbit serum) 1 individual improved symptomatically; 2 objectively; 7 revealed no change and 4 became worse.

Comment. Of the 3 patients showing definite objective improvement after injection of ACS, 1 presented a problem in diagnosis; differentiation between rheumatic fever and early rheumatoid arthritis could not be definitely made. The patient was 31 years of age; there was a history

of "rheumatic fever" 3 years previously when he had fever and inflammation of many extremity joints. The illness subsided spontaneously but recurred the following year. He had been treated with vaccines and Roentgen ray therapy to the spine for early spondylitis rhizomelique. There was roentgenologic evidence of sacroiliitis bilaterally. When first seen in the clinic, he stated that his knees had been swollen, painful and stiff for several weeks. The wrists, fingers and ankles had been stiff. Examination revealed that the patient had a temperature of 102° F. The knees were inflamed, tender and swollen with excessive synovial fluid. There was no loss of passive motion in the wrists, fingers or ankles although at the extremes of motion there was pain. Laboratory findings included erythrocyte sedimentation rate—39 mm. per hour (Westergren): white blood count 6900 with 76% polymorphonuclears, 22% lymphocytes and 2% eosinophils. The trypan blue index before ACS therapy was 13. The patient was treated with a series of 4 injections of ACS increasing from 0.25 to 1.25 cc. following which the joints were slightly more comfortable; there was little change objectively. The sedimentation rate rose to 42 mm. per hour; the white blood cell count fell to 6300 with 67% polymorphonuclears, 31% lymphocytes and 2% eosinophils. The trypan blue index remained 13. After 3 weeks a similar series of ACS injections were given. The patient felt so well that he neglected returning to the clinic. When seen 4 months later he stated that his joint pains and swelling had completely subsided 3 weeks after the last injection. On examination there was no evidence of joint disease. His sedimentation rate at this time was 10 mm. per hour, the leukocyte count was 7500 with 66% polymorphonuclear cells, 32% lymphocytes, 1% monocytes and 1% eosinophils.

The second patient, age 62 years, had had rheumatoid arthritis for 18 years. He had been treated in the clinic with physical therapy, gold salts, vaccines and high dosage vitamin D orally. He did not improve, so agreed to enter the hospital for further treatment. He was brought to the hospital in a wheel chair, unable to walk. There was advanced involvement of the knees, hips, spine, hands, shoulders and feet. The erythrocyte sedimentation rate was 136 mm. per hour. The patient was given a course of ACS injections ranging up to 2 cc. There were no local reac-

TABLE 4.—EVALUATION OF ACS AND NORMAL RABBIT SERUM USED IN TREATMENT OF PATIENTS WITH ARTHRITIS

	Symptomatic improvement	Objective improvement	No change	Worse
	Antireticular Cytotoxic Serum			
Rheumatoid arthritis:				
Early	1	1*	1	0
Mod. advanced	0	0	5	3
Advanced	0	1	6	2
Juvenile	0	0	0	1
Spondylitis:				
Mod. advanced	1	0	0	0
Advanced	1	1	4	1
Totals (29 cases)	3 (10.2%)	3 (10.2%)	16 (55%)	7 (24%)
	Control Rabbit Serum			
Rheumatoid arthritis:				
Adult	1	2	6	4
Juvenile	0	0	1	0
Totals (14 cases)	1 (7%)	2 (14%)	7 (50%)	4 (29%)

* Diagnosis not certain—? rheumatic fever.

tions. Upon completion of this series there was a marked subjective and objective improvement, the joint synovitis subsided. The patient began to walk. The sedimentation rate reduced to 122 mm. per hour. A second course of injections of ACS was given 2 months later and a third 1 month after that. The patient continued to improve, joint inflammation lessened and there was increased range of motion. The sedimentation rate fell to 106 mm. per hour. Examination 1 year later revea ed that the improvement was sustained.

The third patient was a man 27 years old with advanced ankylosing spondylitis of 8 years duration. He had received Roentgen therapy over the back and also had been treated with nicotinic acid. He came to the clinic requesting ACS therapy. Motion of the spine was greatly restricted and chest expansion was only $1\frac{1}{8}$ inches. Forward bending while standing with the legs straight was so reduced that the fingertips were 24 inches from the floor. The erythrocyte sedimentation rate was 83 mm. per hour; leukocyte count 7400; there were 64% polymorphonuclear cells, 30% lymphocytes and 6% eosinophils. The first series of 3 ACS injections ranged from 0.5 to 1.5 cc. There was a marked tissue reaction at the site of injection accompanied by systemic fever of 101.2° F. following the last injection. A second series of injections was given 4 weeks later. There was no local reaction at this time. Five weeks later ACS was given in a third series of injections where again there was marked local reaction. From the beginning of the treatment the chest expansion increased from $1\frac{1}{8}$ to $1\frac{7}{8}$ inches and the finger to floor measurement improved from 24 to 14 inches. The erythrocyte sedimentation rate gradually fell to 39 mm. per hour. The patient stated he felt much stronger, had no back pain, was less handicapped getting in and out of an automobile and could carry a suitcase for the first time in many years. When reëxamined 2 months later it was found that the improvement had been maintained. At this time the patient admitted that throughout the course of ACS therapy he had been doing daily breathing exercises and calisthenics which he had overheard were advised to other patients.

Observation over a period of several months showed the symptomatic improvement experienced by 3 patients to be only temporary.

Discussion. Evaluation of any method of therapy for arthritis is difficult indeed. The natural changes in the course of the disease leading to improvement must always be considered. No effort was made in this study to evaluate the psychogenic factors which are known to influence the progress of rheumatoid arthritis. The improvement noted in the third case may be assumed to be due in part to exercises. The fact that objective improvement occurred in 2 patients of the control group suggests that benefit observed in the ACS treated cases may not be due to the antireticular cytotoxic characteristics of the serum but rather to a non-specific serum effect.

There have been relatively few studies of ACS used in the treatment of rheumatism. Bach[1] treated 48 cases, 32 hospitalized patients and 16 clinic patients. Besides rheumatoid arthritis the illnesses treated with ACS included rheumatic fever, gonorrheal arthritis, osteoarthritis and non-articular rheumatism. Clinical improvement was noted in 14 cases (29%); in 7 patients improvement was classified as "definite," in 7 others "slight." Benefit was mainly subjective and in many cases relapses occurred after a short time.

Conclusions. 1. Polyarthritis induced in rats by pleuropneumonia-like organisms was not affected in time of onset or severity by the use of antireticular cytotoxic serum either prophylactically or therapeutically.

2. With the method of administration used in this study ACS therapy was of no benefit in the great majority of patients with rheumatoid arthritis. In the few cases apparently improved, benefit was usually transient. There was no dependable effect of ACS on the clinical course of rheumatoid arthritis.

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GLYCOGEN STORAGE (VON GIERKE'S) DISEASE PREDOMINANTLY INVOLVING THE HEART

Report of a Case With Histochemical Phosphatase Studies

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Enlargement of the heart due to abnormal glycogen storage is of rare occurrence. The present communication deals with a case in which large amounts of glycogen were demonstrated in the heart and other organs by several histochemical methods. In addition, the distribution of alkaline and acid phosphatase in sections of various organs was investigated. Such a study seemed of particular interest in view of the findings of Thannhauser, Sorkin and Boncoddo.[36] These authors reported on chemical examinations a marked decrease in alkaline phosphatase activity in the livers of 3 patients who died of the hepatic form of von Gierke's disease.

Case Abstracts. The patient was a female child of Polish-Russian extraction. There was no consanguinity between the parents. The mother had given birth to 3 children, at the age of 19, 20 and 22. These 3 children are healthy and developing well. At the age of 25 this female child to be reported was born. The delivery was without difficulty. The infant did not take food well and did not gain weight. It also had frequent loose, foul smelling bowel movements. At the age of 6 weeks the child was admitted to the Elizabeth A. Horton Memorial Hospital in Middletown, N. Y., under the care of Dr. A. Romain. Physical examination revealed a poorly nourished, dehydrated baby weighing 3910 gm. The temperature was normal. The blood count was essentially normal. Several urine examinations revealed absence of sugar and acetone. Upon treatment with saline infusions, and pectin agar, the baby gained 430 gm. in 9 days. The child appeared to be markedly improved upon discharge. At home she failed, however, to gain further weight and did very poorly. Four weeks later the mother noticed in the morning that the child was cyanotic and dyspneic. It was dead upon admission to the hospital a few hours later. An autopsy was carried out 20 hours after death.

Necropsy. The general appearance of the body was that of a 10 weeks old female baby in a very poor nutritional state. The skin was dry and the eyes sunken in. No edema or jaundice was noticed.

The abdominal cavity did not contain any free fluid. The liver was one finger beneath the costal margin. In the right pleural cavity a few cc. of free fluid was found. The heart was very considerably enlarged and weighed 99 gm. (normal 23 gm.)[7] (Fig. 1.) The pericardial sac did not contain any increased amount of free fluid. The pericardium was smooth. Dissection of the heart showed no congenital abnormalities. The foramen ovale was closed. There was marked hypertrophy of the ventricles, the left measuring up to 2 cm. and the right up to 0.7 cm. in thickness. The myocardium, on cut section, had a grayish, pale color. The papillary muscles were very markedly hypertrophied. The endocardium was smooth and pale. The aorta and coronary arteries appeared normal. The lungs weighed 130 gm. The right upper and portions of the middle and lower lobe were hypo-aerated and dark red in color. The left lung contained small patchy areas of hypo-aeration. The mucosa of the bronchi was pale.

The liver was moderately enlarged and weighed 220 gm. (normal 140 gm.).[7] The outer surface was smooth and brownish. The section oozed a good amount of dark red blood and had a fairly uniform reddish appearance. The spleen was not enlarged and weighed 15 gm. On cut section it was purplish and showed the anatomical markings well. The adrenals appeared to be somewhat atrophic. Thymus and pancreas were not unusual on gross examination.

The kidneys were not enlarged, each

weighing 20 gm. On cut section they appeared normal. The remaining organs of the G. U. tract and of the G. I. tract appeared grossly normal. Sections through lumbar vertebræ showed moist red bone marrow. The brain appeared grossly normal.

MICROSCOPIC EXAMINATION: Tissues were fixed in 20% formalin and sections stained with hematoxylin-eosin. Pieces from heart, adrenals, kidneys and liver were in addition fixed in absolute alcohol and in cold acetone. Glycogen was demonstrated with Best's carmine stain and the Bauer-Feulgen stain,[4] as well as by Gomori's modification of Bauer's technique.[17]

Heart. Sections through many areas of the heart stained with hematoxylin-eosin appear similar throughout (Fig. 2). The muscle fibers are hypertrophied and reveal vacuolization. In many muscle fibers these vacuoles appear as large empty spaces. On cross-section these empty spaces are surrounded by a narrow ring of pink staining sarcoplasm. The nuclei are mostly situated at the periphery. Occasionally they appear

FIG. 1.—The left ventricle of the heart is opened. Hypertrophy of the wall and papillary muscles is conspicuous.

Satisfactory staining for glycogen was possible not only in alcohol and acetone fixed tissue but also after fixation in 20% formalin.[3]

Alkaline phosphatase was demonstrated with Gomori's technique[12] in the modification of Kabat and Furth.[20] The slides were incubated for 12 to 14 hours in the substrate mixture. No counterstain was used. Acid phosphatase activity was revealed with Gomori's technique[13] as modified by Wolf, Kabat and Newman.[43] The incubation time varied from 4 to 48 hours. No counterstain was employed. Lipase activity was demonstrated with Gomori's stain[16] with some modifications.[41]

to be in the center. Glycogen stains reveal the intracellular spaces in the muscle fibers to be filled by fine and coarse granules and rods (Fig. 3). Occasional glycogen granules appear to be lying outside the muscle fibers. No glycogen is present in the nuclei.

Alkaline Phosphatase: There is slight activity in the nuclei of muscle fibers and faint in the remaining remnants of the sarcoplasm. The capillaries and small arteries show very marked phosphatase activity.

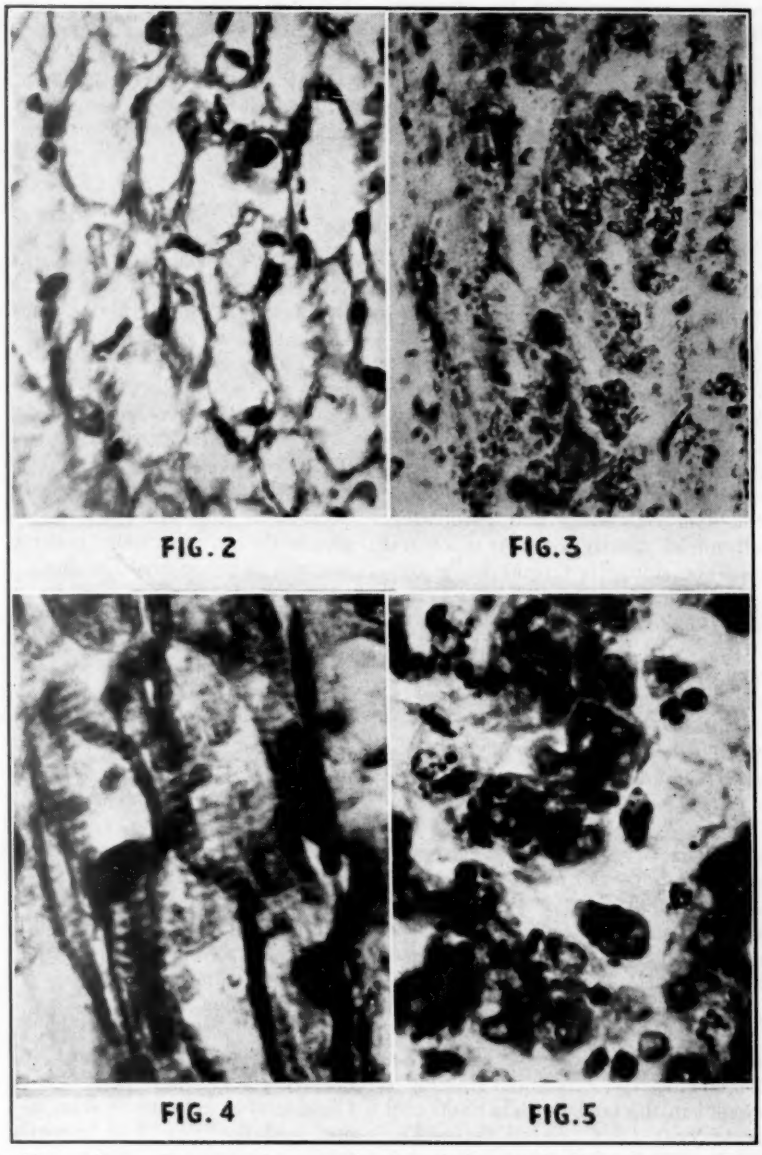

FIG. 2.—Myocardium. The extensive vacuolization of the muscle fibers is seen. Hematoxylin-eosin. × 400.

FIG. 3.—Myocardium. Best's carmin stain for glycogen. The droplets filling the muscle fibers correspond to the red staining. Glycogen. × 400.

FIG. 4.—A section of myocardium stained for acid phosphatase. The sites of enzymatic activity stained dark. Nuclei and cross-striations show enzymatic activity. × 900.

FIG. 5.—Liver. Bauer-Feulgen stain for glycogen. The dark droplets filling the liver cells represent glycogen. Several Küpffer cells located in the dilated sinusoids are loaded with glycogen. × 540.

Acid Phosphatase (48 hours incubation): There is distinct phosphatase activity in muscle fibers not occupied by glycogen. The striations are very prominent (Fig. 4). There is considerable activity in the nuclei of the muscle fibers as well as of those in the connective tissue and vessel walls.

Lungs. Section through grossly changed areas show considerable thickening of alveolar septa due to infiltration by mononuclear cells as well as occasional polymorphonuclear leukocytes. In addition considerable congestion of capillaries is present. There is exudate found in many alveolar spaces composed of large alveolar cells, smaller mononuclear cells, and occasional polymorphonuclear leukocytes. Glycogen is demonstrable in the smaller bronchi in the epithelium, smooth muscle cells and within the cells of cartilage. Many of the large alveolar cells contain glycogen granules.

Liver. Hematoxylin-eosin stained sections show the liver cells large and vacuolated. The cell nuclei mostly occupy a central position. The sinusoids are markedly congested and occasional Küpffer cells are swollen and detached from the wall. Sudan stain on frozen section reveals only a very moderate amount of fat droplets in most liver cells. Glycogen stains demonstrate large amounts of glycogen in the liver cells. The glycogen forms small and coarse granules and rods. Many of the Küpffer cells contain considerable amounts of glycogen (Fig. 5).

Alkaline Phosphatase: Nuclei, chromatin and nuclear membranes show a fair amount of alkaline phosphatase while the cytoplasm contains only very little. The activity of the sinusoidal endothelium varies in different fields. Bile capillaries are not stained (Fig. 6).

Acid Phosphatase (4 hours incubation): Considerable activity in nuclei and cytoplasm is seen (Fig. 7). Prominent staining is also apparent in the nuclei of bile ducts and in the nuclei located in the vessel walls. The Küpffer cells exhibit acid phosphatase activity. When the incubation period was extended to 24 hours complete darkening of the section occurred obscuring all cellular details.

Lipase Activity (48 hours incubation): Considerable lipase activity is present, localized exclusively in the cytoplasm of the liver cell cords.

Kidney. No significant changes are seen in sections stained with hematoxylin-eosin. Glycogen stains, however, reveal a considerable amount of glycogen in the distal convoluted tubules, Henle's loops and collecting tubules. The proximal convoluted tubules are apparently free. Some glycogen is also present in the glomerular epithelium (Fig. 8).

Alkaline Phosphatase: Conspicuous phosphatase activity is seen in the cytoplasm of the proximal convoluted tubules. It is most prominent at the brush borders. All other tubules show phosphatase activity only in the nuclei. Similarly the nuclei in the glomeruli show phosphatase activity. Some small arteries in the cortex as well as capillaries in the medulla are in addition the site of distinct enzymatic activity (Fig. 9).

Acid Phosphatase (48 hours incubation): There is cytoplasmic phosphatase activity in most of the tubules located in the cortical portion of the kidney. In addition the glomeruli are distinctly positive. In the medulla only the nuclei of the tubules show phosphatase activity. While after a 4 hour incubation period practically no staining is seen, slides incubated 24 hours show similar, although less marked staining as slides after 48 hours incubation.

Adrenals. Hematoxylin-eosin stained sections show considerable congestion of the medulla. Glycogen stains reveal a good amount of glycogen in the cortical cells and large amounts in the cells of the medulla. Occasional glycogen granules are present in the periadrenal fat tissue.

Alkaline Phosphatase: There is considerable amount of phosphatase activity in the nuclei and cytoplasm of the cortical cells. The zona glomerulosa stains less intensely than the zona fasciculata and reticularis. The medullary cells contain a fair amount of alkaline phosphatase (Fig. 10).

Acid Phosphatase (4 hours incubation): Considerable activity is seen in the cortex and medulla (Fig. 11). In sections stained for a longer time the intense staining reaction obscures all cellular detail.

Pancreas. The Langerhans islands are numerous and some of them very large. In sections stained with Best's carmin, the Langerhans islands are outlined as red areas due to the large amount of glycogen which they contain. In contrast the acinar cells contain only traces. Large amounts of gly-

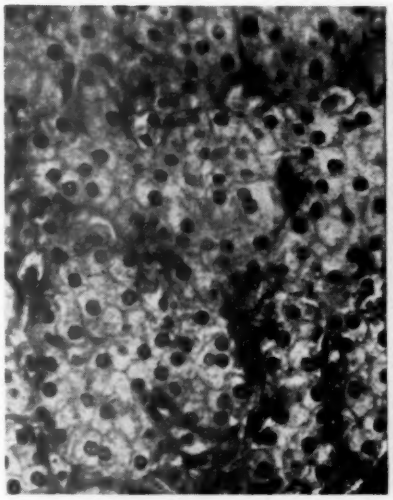

FIG. 6

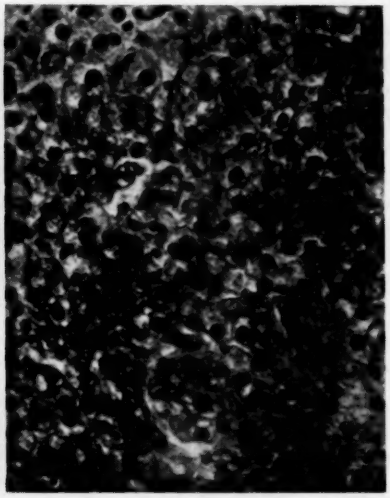

FIG. 7

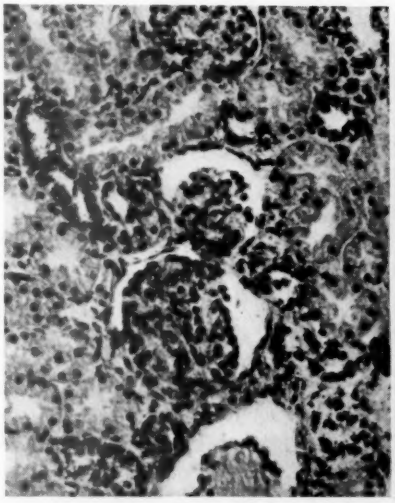

FIG. 8

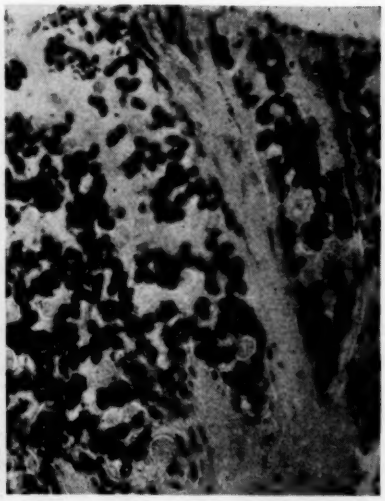

FIG. 9

FIG. 6.—A section of liver stained for alkaline phosphatase. The sites of enzymatic activity stain dark. Nuclei and sinusoids are stained while the cytoplasm shows only little staining. × 300.

FIG. 7.—A section of liver stained for acid phosphatase. The sites of enzymatic activity stain dark. Nuclei and cytoplasm of liver cells reveal acid phosphatase activity. × 300.

FIG. 8.—Kidney. Best's carmin stain for glycogen. Glycogen granules are present in the capsular epithelium of the glomeruli and in distal convoluted tubules. × 400.

FIG. 9.—A section of kidney stained for alkaline phosphatase activity. The sites of enzymatic activity stain dark. Alkaline phosphatase is present in the proximal convoluted tubules. × 30.

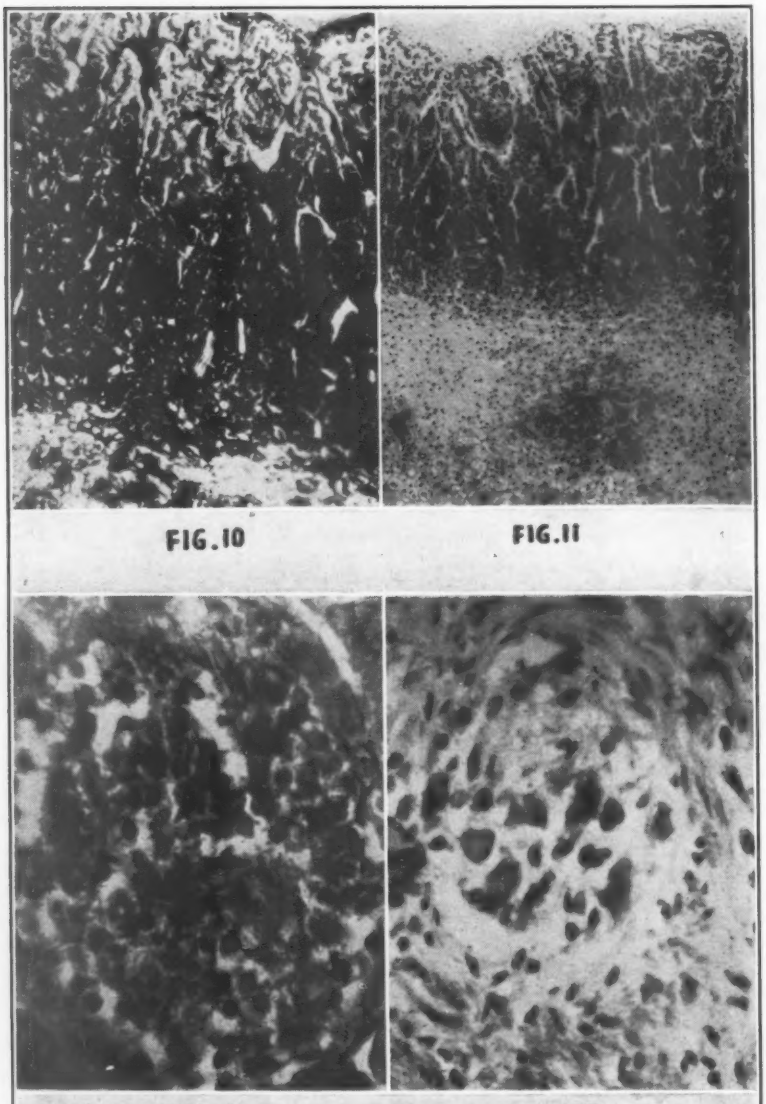

FIG. 10.—A section of adrenal stained for alkaline phosphatase. The sites of enzymatic activity stain dark. Alkaline phosphatase activity is present predominantly in the zona fasciculata and reticularis. × 140.

FIG. 11.—Section of adrenal stained for acid phosphatase activity. The sites of enzymatic activity stain dark. Acid phosphatase is present in the cells of the cortex and medulla. × 100.

FIG. 12.—An enlarged Langerhans island of the pancreas. Chromium-hematoxylin-phloxin stain. The dark granules in many of the cells correspond to the blue staining beta cells. × 450.

FIG. 13.—Myenteric plexus in the large intestines. Best's carmin stain for glycogen. The granules in the nerve cells represent glycogen. × 300.

cogen are also present in the cells of the larger ducts. In sections stained with the Gomori chromium hematoxylin-phloxin stain,[13] the majority of the cells in the Langerhans islands stain blue and therefore represent beta cells (Fig. 12).

Spleen. In contrast to the lymphocytes some of the large mononuclear cells contain glycogen.

Lymph Nodes and Thymus. Occasional reticulo-endothelial cells contain glycogen granules, while the lymphocytes are devoid of them.

Intestine. Smooth muscle cells contain a large amount of stainable glycogen. In sections stained with Best's carmin, the muscularis mucosa stands out as a red colored band. The nerve cells of the myenteric plexus of Auerbach contain large amounts of glycogen (Fig. 13).

Diaphragm. In sections stained with hematoxylin-eosin most of the muscle fibers appear normal, while occasional ones have a somewhat vacuolated appearance. Specific stains reveal that these vacuoles are due to glycogen infiltration. The other muscle fibers contain occasional small glycogen granules.

Brain. Many cells in various sections from the brain contain glycogen in forms of fine cytoplasmic granules. The glia cells appear to be more involved than the ganglion cells.

Blood-vessels. In all organs many of the blood-vessels contain glycogen. It is found in the muscle fibers as well as in the endothelial cells.

Discussion. In glycogen storage disease there exists apparently a developmental defect involving the enzyme systems necessary for the transformation of glycogen to dextrose and of dextrose to glycogen. The various metabolic aspects of this disease have been recently discussed by Mason and Anderson,[25] Kato,[21] and Bridge and Holt.[6]

While in the more common form of Von Gierke's disease, the liver and often the kidneys are the main site of glycogen storage, in some of the cases the heart is the site of grossly abnormal glycogen deposition. Review of the literature reveals 17 cases in which the presence of grossly abnormal amounts of glycogen was demonstrated in the heart muscle without other congenital abnormalities either by histochemical methods or chemical analysis (Sprague, Bland and White,[35] later proven as a typical case;[19] Pompe, 3 cases;[30] Putschar;[32] Humphreys and Kato, 3 cases;[19] Antopol, Heilbrunn and Tuchman;[2] Hertz and Jeckeln;[18] Wolff;[44] Mutgeert;[27] Gardner and Simpson;[10] van Greveld;[37] Antopol, Boas, Levinson and Tuchman;[1] Bonaba, Saldun de Rodriguez and Ferreira Beruti;[5] Scheidegger;[33] and Schneider.[34])

In addition, in these patients the liver showed also a varying degree of grossly demonstrable enlargement. Kimmelstiel's case occupies probably an intermediate position.[22] In this case the liver was enormously enlarged due to deposition of glycogen, while the heart muscle revealed glycogen infiltration on microscopic examination without being distinctly hypertrophic. Most of the children with the cardiac form of glycogen disease died before reaching the 1st birthday. Only 2 patients were reported which attained an age of 11 and 15 years respectively.[1,10]

In the present case glycogen was found not only in the heart and liver but also in many other locations on microscopic examination. This is in full accord with the cases reported in the literature in which a varying amount of glycogen infiltration in different organs occurred. While most investigators employed Best's carmin stain for the demonstration of glycogen under the microscope, use was made in addition of the Bauer technique in its original form and as modified by Gomori. As Gomori[17] has pointed out, these methods constitute a more specific staining reaction based on known chemical principles than the purely empirical Best's stain.

In the liver not only the hepatic cells proper but also the Küpffer cells showed large amounts of glycogen. Cells belonging to the reticulo-endothelial system in lymph nodes, spleen and thymus also contained glycogen, although less regularly. Only in the case of Wolff[44] were appreciable amounts of glycogen found in Kupffer cells and reticulo-endothelial elements of the spleen. In all other reports in which

mention is made of the Kupffer cells they were found either to be devoid of glycogen or contained only occasional traces. While, therefore, the reticulo-endothelial system and particularly the Kupffer cells do not often participate in the glycogen storage, the present case as well as Wolff's report do not permit the conclusion that in contrast to Gaucher's and Pick-Niemann's disease the reticulo-endothelial system in von Gierke's disease is not involved by the abnormal cell metabolism.[2,24]

No regular distribution within the tubular segments is seen in the kidneys of cases with glycogen disease. This has been pointed out by Wolff[44] and von Gierke.[39] In contrast, in the diabetic kidney the glycogen is found in the terminal segment of the proximal convoluted tubules and to a lesser degree in the ascending limbs of Henle's loop.[28] With the maceration and dissection technique, Oliver found no localization to any particular part of the nephron in von Gierke's disease, for collections of glycogen containing cells are scattered irregularly throughout all its parts.[29]

Glycogen deposition in the brain has been described in several instances.[22,33,34] In the present case in addition cells in the nervous plexus of the intestinal tract participated in the abnormal storage. In cases of amaurotic family idiocy (Tay-Sachs disease) Globus[11] found abnormal lipoid material not only in the central nervous system but also in the nerve cells of the abdominal viscera, including the gastro-intestinal tract.

The histochemical distribution of alkaline and acid phosphatase in the tissue sections in heart, liver, kidney and adrenals was not significantly different from that seen in sections from human organs not involved by disease.[13,14,20,42,43] The results of these staining reactions do not support the view put forth by Thannhauser and co-workers,[36] that in glycogen storage disease there is deficiency in alkaline phosphatase. It is of interest to note that Bridge and Holt,[6] by incubating liver slices, from a biopsy of a patient afflicted with von Gierke's disease with muscle and liver phosphorylase preparations, observed only little breakdown of the stable glycogen.

The changes noticed in the pancreas deserve some comment. While in a number of reports no mention is made of the pancreas, no significant changes were recorded by Humphreys and Kato, in 3 cases of the cardiomegalic type[19] and by Hertz and Jeckeln[18] and Antopol and co-workers[1] in another case of this type. Von Gierke stressed the absence of changes in the Langerhans islands in 2 cases of the hepatomegalic type.[38,40] On the other hand, in 2 cases of the atter type described by Krakauer[23] and in a similar case reported by Esser and Scheidegger[8] the islands were found to be large and abundant. Similarly enlarged islands were seen in the case of the cardiomegalic type observed by Scheidegger[33] and Schneider[34] and in the case of Kimmelstiel.[22] Faber in a case of the hepatomegalic type found the number of islands diminished and some very large.[9]

The significance of the hyperplastic changes in the islands of Langerhans is difficult to evaluate. Changes of this kind are found in newborn babies from mothers with diabetes[31] and those who develop signs of diabetes mellitus later[26] as well. It is also sometimes found in infants suffering from erythroblastosis.[31] However, hyperplasia of the Langerhans islands occurs occasionally in infants without apparent reasons.[31]

It was observed by us, for instance, in the pancreas of an infant who died at the age of 3 months of congenital heart disease. The chromium hematoxylin-phloxin stain revealed only a small proportion of the cells in the hyperplastic islands to be of the beta type, while in the case of glycogen disease the majority of cells were of the beta type. Although no conclusion can be drawn from this single observation, a study of the type of cells forming the hyperplastic islands in newborn infants under various conditions seems very desirable.*

* Recently such a study was carried out in 3 cases of congenital hyperplasia of the pancreas in fetuses of diabetic mothers. An increase in beta cells in proportion to the number of alpha cells was found. (Hultquist, G. T., Lindgren, I., and Galgaard, J. B.: Nordisk. Med., **31**, 1841, 1946.)

In view of the fact that hyperplasia of the Langerhans islands appears to occur fairly frequently in glycogen storage disease, one might speculate as to a possible connection with increased demand for insulin.

Ingestion of carbohydrates provokes in many patients with von Gierke's disease an abnormally high and persistent rise of blood sugar. It is possible that the hyperplasia of beta cells indicates increased insulin production called upon to depress the increased amount of the sugar in circulation.

Summary. The 18th proven case of von Gierke's disease with marked enlargement of the heart is described. On microscopic examination glycogen storage was demonstrable in the cells of various organs, including the brain, the nerve cells in the myenter c plexus of the intestinal tract and the Küpffer cells of the liver. The histochemical distribution of alkaline and acid phosphatase in liver, kidney, adrenal and heart was found to be normal. Marked hyperplasia of the Langerhans islands in the pancreas with preponderance of beta cells was present. The significance of these findings is discussed.

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STUDIES OF CRYOGLOBULINS

I. Unusual Purpura Associated with the Presence of a High Concentration of Cryoglobulin (Cold Precipitable Serum Globulin)*

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The literature contains but sparse reference to cases of purpura associated with cold precipitable serum globulins. There are only 9 reports of proteins precipitating spontaneously from serum (usually cooled).[2,3,4,6,9,11,13,15,17] Purpura was observed in 2 of these cases.[15,17]

There are 3 reports on the association of purpura and hyperglobulinemia.[1,10,14] In 1 of the papers,[10] a viscous serum protein is reported.

The purpose of the present paper is to report an unusual case and discuss some of its implications. The chemical characteristics of the protein observed in this case have been described in detail elsewhere.[7]

Case Report. J. P., a white male, age 56; No. 726624. This patient was first admitted to the University Hospital on Dec. 1, 1942, and discharged on Jan. 30, 1943. He was readmitted on Oct. 15, 1943, and expired in the hospital on Feb. 20, 1944.

In the fall of 1934 the patient experienced a sharp pain across the epigastrium. The pain extended up to just beneath the costal margin. A dull pain over the liver area was associated with this sharp pain. He was not jaundiced. The distress persisted for several days, then disappeared.

In the summer of 1935, after swimming in a lake for 10 minutes on a warm day the patient felt chilly, weak and faint. Hives developed over most of his body. By the next morning the hives disappeared. A few months later, in the fall, he noticed a red, pin-point flat rash on his ankles. "Red blisters" formed, which later turned black and finally fell off, leaving a pigmented area. He began to notice that he was very sensitive to cold. A cold wind or exposure to cold of any sort produced pain and swelling in the exposed parts. It was necessary for him to wear heavy underwear and an unusual amount of clothing to keep warm.

He experienced another attack of sharp pain across the epigastrium in April 1937. This lasted for about 1 week and was similar to the first one. Again there was no jaundice.

In 1937 the purpuric skin lesions extended up to and included the knees. The lesions remained unchanged until 1939. At this time they began to spread again and involved the ears, thighs, abdomen, buttocks and scrotum. He was not able to secure any adequate medical explanation for these lesions.

The patient was studied at the Mayo Clinic in March and July of 1942.‡ At that time he complained of inability to work because of swelling of his feet accompanied by a burning and stinging pain in the legs at the site of the subcutaneous hemorrhages. This condition was markedly aggravated by prolonged standing. He was unable to wear ordinary shoes for over a year and instead wore slippers and overshoes.

The past medical history, except for smallpox as a child, was non-contributory. There was no history of food allergy, hay fever or asthma. His father died of uremia at age 61. His mother died of "old age" at 78 years. He had 2 brothers and 5 sisters. A brother

* A portion of this study was included in a thesis on the plasma proteins, submitted by Dr. Lerner to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Doctor of Philosophy degree.

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‡ We are indebted to Drs. Bayard Horton and Byron Hall for the information obtained in July 1942.

5 years younger has an enlarged heart and prostate. One sister is a diabetic.

The patient was happily married since 1917 and had 3 children, all living and well. Until 1934 he was a broom manufacturer and real estate agent. In 1934 he began working in a hard-rock mine in Montana, where he was exposed to ore containing lead, zinc, copper, silver, gold, bismuth, arsenic and sulfur.

On physical examination in July 1942, the blood pressure was found to be 108/72. The pulse and temperature were normal. There was marked narrowing and sclerosis of the retinal arterioles, but there were no retinal hemorrhages. Examination of the lower extremities revealed extensive subcutaneous hemorrhages of various age, the majority being old. He had edema of the feet and ankles. There was considerable brownish pigmentation of the skin of the lower legs. The purpuric lesions were also present on the ears, abdomen, buttocks and scrotum.

The laboratory findings in July 1942 were as follows: Urinalysis and serologic tests for syphilis were negative. The hemoglobin was 13.8 gm. %. The blood platelets were 118,000, 201,000 and 196,000 per c.mm. on 3 successive occasions. Blood smears were negative. The bleeding time, clotting time, clot retraction and prothrombin time were well within normal limits. The blood urea nitrogen was 32 mg. per 100 cc. Fasting blood sugar, cholesterol, phosphatase, ascorbic acid, serum bilirubin, calcium and phosphorus were all within normal limits. The basal metabolic rate was −5. The bromsulfalein liver function test showed a "Grade 1" dye retention. Gastric analysis revealed a total acidity of 48 units with free hydrochloric acid of 30 units; the total quantity was 150 cc. A chest Roentgen ray film was negative.

The diagnosis in 1942 was "cold allergy." The patient was desensitized to histamine phosphate and given vitamin P, but without apparent relief.

About 4 months later, on Dec. 1, 1942, he was admitted to the University of Minnesota Hospital. He complained of severe pain in the left lower chest, accompanied by dyspnea, cough, blood-streaked sputum and fever. These symptoms were noted first about 2 months previously. He had had severe attacks of precordial oppression and dyspnea on November 3, 13 and 30. After that time, he had precordial distress upon exertion. The purpuric lesions on the extremities were increasing.

In December 1942 the blood pressure had increased to 156/94. The temperature was 98.4°, pulse 90 and respiration 20. There was slight dullness, diminished breath sounds and diminished tactile fremitus at the base of the left lung posteriorly. The heart was slightly enlarged to the left, cardiac rhythm was regular and no murmurs were heard. The liver was palpable 2 cm. below the right costal margin in the midclavicular line. There was a slight pitting pretibial edema. A finely mottled brown pigmentation was noted on the ears, hands, wrists, feet, legs, thighs and about the waist. There were fresh petechiæ on both legs.

Urinalysis revealed a 1+ to 2+ albumin with an occasional leukocyte. The hemoglobin was 11.2 gm. per 100 cc. of blood. The leukocyte count was 10,900, 64% neutrophils, 26% lymphocytes, 1% basophils and 9% eosinophils. Serologic tests for syphilis were negative. The blood urea nitrogen was 25 mg. %. There was only a trace of serum ascorbic acid. The prothrombin time and coagulation time were normal. Bleeding time was 7.5 minutes. The cuff test was positive. The platelet count was 165,000 per c.mm. Sedimentation rate was 36 mm. in 1 hour (Westergren method). The vital capacity was 2000 cc., and the venous pressure was 13.5 cm. of water.

A chest Roentgen ray revealed generalized cardiac enlargement, a tortuous aorta, bilateral congestion of the lungs, and a small amount of fluid in the left pleural cavity. A roentgenkymogram showed no evidence of myocardial infarction. Electrocardiographic studies revealed a pattern suggesting left ventricular strain. A biopsy of the skin showed what was interpreted as a "capillaritis." This consisted of moderate round cell infiltration about the capillaries in the purpuric areas. A deltoid muscle biopsy gave negative findings.

The patient was confined to bed and given 100 mg. of vitamin C twice daily. It was found that he was sensitive to moccasin snake venom, and attempts made to desensitize him were without benefit. His cardiac condition gradually improved, and the venous pressure fell to 7.5 cm. of water. He was discharged on Jan. 30, 1943, and advised to take 500 mg. of hesperidin 4 times daily.

He was to be followed in the Out-Patient Clinic. The diagnosis at this time was idiopathic non-thrombocytopenic purpura, coronary arteriosclerosis and essential hypertension.

On Oct. 15, 1943 he was again admitted to the hospital. He complained of extensive purpuric skin lesions, generalized edema which had been more severe during the week prior to admission, together with periods of irrationality, and an oliguria of less than 500 cc. during the 24 hours prior to readmission. He was bedridden for over a year because of the purpura, edema and pain which shifted over his body. He had frequent nosebleeds. One week prior to admission he was seen in the Out-Patient Clinic and put on a diet of milk and water. During this time his edema and general condition became much worse.

His blood pressure was now 182/106. He was markedly orthopneic, and there was severe generalized edema involving the legs, abdomen and lower chest. The skin of the extremities was covered with old and fresh petechiæ. These were so extensive over the fingers that the patient preferred to protect them by bandages. Hemorrhages were also present over the scrotum, but the trunk was uninvolved. The skin over the feet and legs was indurated and bronze to blue-black in color. A biopsy revealed extensive hemosiderosis.

Funduscopic examination was not satisfactory. A few râles were heard at the lung bases posteriorly. The cardiac tones were clear and regular. No murmurs were heard. There was a marked ascites and satisfactory palpation of the abdomen was impossible.

Urinalysis showed a 1.014 to 1.020 sp. gr. with a consistently 1+ to 3+ albumin and occasional leukocytes, red blood cells and casts. Hemoglobin was 7.9 gm. per 100 cc. Erythrocytes were 2,600,000 per c.mm. The leukocytes ranged from 6000 to 16,000, with a predominance of neutrophils. The blood urea nitrogen rose from 28 to 36 mg. per 100 cc. Plasma proteins were 5 and 5.3 gm. % with albumin 3.5 and globulin 2.3 gm. %. Sedimentation rate was 122 mm. in 30 minutes. Hematocrit reading was 22.8%. Reticulocytes were 0.8%. Platelets were 410,000 per c.mm. Serologic tests for syphilis were negative. Bleeding time, clot retraction and clotting tme were within normal limits. Cephalin-cholesterol flocculation test was negative. There was no Bence-Jones protein in the urine. Cuff test was positive. Venous pressure was 9.5 cm. of water. Vital capacity was 2000 cc. When the blood serum was allowed to stand in the refrigerator, a flocculent material, which had an oily appearance, separated out. This substance would dissolve when the serum was incubated at 37° and would reform on cooling.

A chest Roentgen ray showed a generalized cardiac enlargement of the left ventricular type with a bilateral pleural effusion. A renal biopsy revealed chronic glomerulonephritis. (Fig. 1.)

The patient slowly deteriorated and finally expired on Feb. 20, 1944.

At autopsy* it was found that the heart weighed 560 gm. and there was marked left ventricular hypertrophy. There was an area of recent pericarditis. The coronary arteries showed moderate narrowing due to arteriosclerosis. There was no evidence of fresh or old coronary thrombosis. The lungs revealed chronic passive congestion; microscopically there were many heart failure cells and some epithelialization of the alveoli. The liver weighed 1900 gm. and showed slight chronic passive congestion. The gall bladder appeared to be normal. The right and left kidneys weighed 135 and 190 gm., respectively. They were pale in appearance and exhibited typical chronic glomerulonephritis, microscopically.

Characteristics of the Serum Protein Precipitating Spontaneously in the Cold. This protein, the characteristics of which have been described in detail elsewhere,[7] is perhaps best designated as a cryoglobulin because of its outstanding characteristic of precipitating spontaneously from cooled serum (cryo from the Greek *kryos*, meaning cold). The use of this new term would perhaps not be justified were it not for the fact that proteins with similar characteristics have been described in 6 other cases recorded in the literature and that similar proteins have been found in serums from patients with a variety of diseases.†

* Performed by Dr. P. V. Heise.

† A study of the association of these proteins with many diseases is presented in Paper II which follows.

The term *cryoglobulin* is thus suggested to represent a group of *proteins* with the common property of precipitating (or gelifying) from cooled serum. This is similar to the use of the term Bence-Jones proteins to represent a *group* of proteins precipitating at 60° C. The cryoglobulins may precipitate at room temperature if they occur in high concentration.

With respect to solubility, ultraviolet absorption spectrum and nitrogen content the protein from the serum of the case we described resembles the γ globulins. However, this protein, unlike the γ globulins, is soluble in water but insoluble in dilute salt solutions. So far as could be determined,[7] the molecular weight and viscosity are greater than those of the γ globulins.

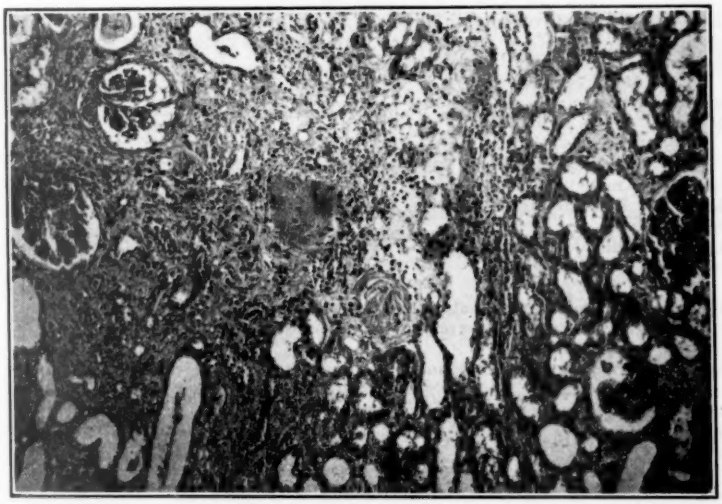

FIG. 1.—Photomicrograph of renal biopsy from Case J. P., to show the extent of the chronic glomerulonephritis on Nov. 24, 1943; hematoxylin and eosin, × 90.

In 0.9% saline at body temperature this protein is very soluble but at room temperature it is almost completely insoluble.* It seems possible that the acropurpura and marked sensitivity to cold could be due to stasis secondary to increased viscosity, or it is even possible that the cryoglobulin precipitates in the capillaries of those areas where the temperature falls significantly below 37° C.

Discussion. As mentioned previously the literature contains but 9 reports of proteins precipitating spontaneously from serum. There are 3 additional references on the association of unusual serum proteins and symptoms similar to those presented by the present case. A brief description of these cases is given in Table 1.†

The cases of Waldenström[13] and Shapiro *et al.*[10] did not show any cold-precipitable proteins and the globulins responsible for the hyperglobulinemia should therefore not be called cryoglobulins. The purpura in these cases, however, may have been due to stasis in the peripheral capillaries on the basis of increased blood viscosity or rouleaux formation as a result of the hyperglobulinemia.[5,8] Anderson and Samuelson[1] did not report whether or not the serum from their patient contained any cold-precipitable proteins. All of the other

* The solubility measurements were made at pH 5.7, the isoionic point of the protein. It is likely that the protein would be more soluble at the pH of blood.

† A case of periarteritis nodosa with cryoglobulinemia and some clinical findings similar to the case described here has recently been reported by B. Shapiro and E. Wertheimer (Brit. J. Exp. Path., **27**, 225, 1946).

reports included in Table 1 specified proteins which precipitated on cooling.

Waldenström speculates that his patients with purpura and hyperglobulinemia may have chronic virus disease.[13,16] He believes that the mechanism of the increased globulin formation is analogous to the predominant formation of virus protein in a plant infected with tobacco mosaic virus. It seems more logical to assume, however, that cryoglobulin formation may be the result of any one of several etiologic agents, *e. g.*, infection (virus and bacteria), malignancy, hepatic cirrhosis, etc.

The reports which are summarized in Table 1 emphasize the association of purpura, or hemorrhagic tendency, with abnormal serum globulins, especially cryoglobulins. Thus "purpura cryoglobulinemica" may be recognized as a sub-group of Waldenström's "purpura hyperglobulinemica," the former being a more qualitative and the latter a more quantitative designation.

The site of formation of cryoglobulins is unknown, but there are 2 indications that they may be produced in the liver. As will be noted in Paper II which follows: small amounts of cryoglobulins appear in some cases of congestive heart failure with enlarged livers. Second, it has recently been noted that the cirrhotic livers of mice poisoned with carbon tetrachloride contain cold precipitable proteins.*

TABLE 1.—CASES SIMILAR TO THE PRESENT INSTANCE EITHER WITH RESPECT TO UNUSUAL SERUM PROTEINS, SYMPTOMATOLOGY, OR BOTH

Observer	Diagnosis	Some of the signs and symptoms	Protein ppt. conc. (gm. %)
Wintrobe and Buell	Multiple myeloma (previously diagnosed as Raynaud's disease)	Cold sensitivity, blanching of skin, peculiar mottling of extremities, nose bleeds, oozing of blood from tongue and gums, thromboses of retinal veins	7.25 (plasma)
Schumacher, Williams and Coltrin	Multiple myeloma	Epistaxis, rectal and vaginal hemorrhage; serum protein "coagulated on exposure to air"	
von Bonsdorff, Groth and Packalen	Multiple myeloma	Bleeding of gums on slight pressure, slight retinal bleeding, congestive heart failure	
Bing	Multiple myeloma	(No information vailable)	
Holmberg and Gronwall	Chronic rheumatic infectious arthritis and spondylitis	(No information available)	1.3 (plasma)
Stein and Wertheimer	Kala-azar (dogs and humans)	(No information available)	0–1.4 (serum)
Atlas, Corden and Bunata	"Liver disease"	(No information available)	
Shapiro, Ross and Moore	Multiple myeloma	Oozing of blood from gums, occasional grossly bloody urine, numerous purpuric spots, occasional vaginal staining	4.2 (serum)
Waldenström	"Incipient myelomatosis or essential hyperproteinemia"	Joint pain, nose bleeds and hyperglobulinemia	
Waldenström	"Purpura hyperglobulinemica"	Chronic purpura of extremities and hyperglobulinemia (3 cases)	
Anderson and Samuelson	(No diagnosis established)	Blue-violet discoloration of hands, feet, nose and ears; "multiple septic emboli and thrombi of retina;" joint pains; hyperglobulinemia	
Flemberg and Lehmann	(No diagnosis established)	Sensitivity to cold, purpura beginning on legs, arms and face which gradually became severe	1.3 (serum)
Lerner and Watson	Cryoglobulinemia and cardiovascular renal disease	Extensive acro purpura, oozing of blood from gums and nose, congestive heart failure	0.8 (serum)

* N. Kretchmer (to be published).

It is of interest to note that the muscle protein, myosin, has solubility properties[12] similar to the cryoglobulin in the serum of the case just described. Myosin, however, has a much smaller molecular weight.

Summary. 1. An unusual case is reported in which purpura and sensitivity to cold were associated with a cold precipitable serum protein.

2. Similar cases previously reported are reviewed and discussed.

3. It is suggested that abnormal globulins may produce purpura, thrombosis, oozing of blood from mucous membranes and sensitivity to cold as a result of their concentration in the blood and more particularly, their unusual solubility properties.

4. The term cryoglobulin is proposed to represent a group of proteins having the common property of precipitating (or gelifying) from cooled serum. If these proteins are in the serum in high concentration, they may precipitate spontaneously at room temperature.

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STUDIES OF CRYOGLOBULINS

II. The Spontaneous Precipitation of Protein from Serum at 5° C in Various Disease States

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The literature contains but few reports dealing with the spontaneous precipitation of protein from serum. Serum turbidity due to precipitated protein may have been mistakenly considered lipid in nature in some instances.

Serum from normal fasting subjects is usually clear. When obtained 1 to 3 hours after a meal, it is generally turbid due to an increase in serum lipids. It has been reported[1,2] that the precipitate noted at times in cases of nephrosis consists largely of cholesterol with a small amount of protein.

When sterile serum is allowed to stand for several months, it usually becomes turbid due to a separation of lipids and protein from the colloidal state.

The present investigation has been concerned with the nature and significance, as well as the rate and degree, of spontaneous precipitation of protein from serum at 5° C. As noted in Paper I, the term cryoglobulin is proposed for the group of proteins having the common characteristic of precipitating in the cold. If the cryoglobulins occur in high concentration they may precipitate from serum at room temperature as in the case of purpura described in Paper I.

Material and Methods. Serums from 121 patients with a variety of pathologic conditions were studied. The serums from 35 conscientious objectors and 5 medical students served as controls. All blood samples were obtained in the fasting state in the morning. The serums were placed in a refrigerator at 5° C. and observed once daily for 6 days to determine the presence or absence of cryoglobulin. One should be careful not to mistake a delayed fibrin clot for cryoglobulin precipitate. A fibrin clot will form as a discrete mass with the appearance of a gel which will not dissolve on warming to 37° C. The cryoglobulin precipitates, however, are composed of small discrete white particles which dissolve when the serum is warmed to 37° C.

If a precipitate formed in the serum, it was separated by centrifugation at 9° C. Further purification was attained by mixing the precipitate with 0.9% saline at 37° C. The precipitate would usually dissolve and reappear when the solution was cooled. This process was repeated twice, and the precipitate was finally dissolved in 0.01 N HCl for the purpose of further studies as noted in the following.

The cephalin-cholesterol test of Hanger[3] and the serum dilution test[8] were carried out on most of the serums studied. According to Hanger and co-workers[4] the gamma globulin fraction is responsible for the flocculation of the cephalin-cholesterol emulsion, the negative reaction in normal individuals being due to inhibition by normal constituents of the albumin fraction. It seemed desirable to ascertain whether there was any correlation between a positive cephalin-cholesterol test and the appearance of a cryoglobulin. The cephalin-cholesterol tests were not carried out on the control serums, which were assumed to be negative.

The serum dilution test is used to deter-

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mine an increase in euglobulins.[8] In this test the serum is diluted 100 times with distilled water. If there is an increase in serum euglobulins, a precipitate will appear in the diluted serum. According to Plotner[8] all serums giving a positive dilution test also give a positive Takata-Ara reaction. The dilution tests were made on most serums (including all control serums) to determine whether or not there was any relation between an increase in euglobulin and the presence of a cryoglobulin.

Table 1.—Occurrence of Cryoglobulin Precipitate in Pathologic Serums Cooled to 5° C

Grade	Cryoglobulin concentration (mg. %)	Time for appearance of precipitate
1	Trace to 6	24 hours—1 week
2	6 to 25	24 hours
3	Over 25	Immediately after cooling*

* If present in high concentration (Grade 3), cryoglobulin may precipitate before the serum is cooled to below room temperature.

Results. The pathologic serums were classified in 3 grades with respect to cryoglobulin precipitates as shown in Table 1.

Spontaneous precipitation of protein occurred in 31 of the 121 cooled serums obtained from the same number of cases of various diseases. Only 1 of the 31 positives was of Grade 3; this was the case described in Paper I. Twelve positive serums were of Grade 2. The remaining 18 positive serums were of Grade 1. The results of further studies of these serums are shown in Table 2. Precipitates were not found in any of the 40 control serums, and the serum dilution tests were negative.

It is of interest that although the cryoglobulins of Grades 1 and 2 were present in small concentrations, they were easily observed in the form of definite white precipitates.

It is evident from Table 2 that there is no correlation between the presence of cryoglobulins in serum and the reaction of the serum to the cephalin-cholesterol and serum dilution tests.

Physical and Chemical Characteristics of the Protein Precipitates. Since only small amounts of cryoglobulins were found in these serums, it was impossible to make detailed studies of the physical and chemical nature of these proteins. It was possible, however, to carry out some studies on 9 protein preparations from Grade 2 serums redissolved in dilute hydrochloric acid (Samples 2 to 10 in Table 2). The findings are given below.

Solubility. All of the protein preparations except No. 9 dissolved when mixed in 0.9% saline at 37° C. On cooling, the cryoglobulin reprecipitated. None of the warm saline solutions, however, was completely clear.

When 0.01 N HCl was used as the solvent, the cryoglobulin dissolved readily and did not reprecipitate when the solutions were cooled at 5° C. However, only the cryoglobulin preparation from Case 9 gave a truly clear solution. The remaining solutions were slightly cloudy. On standing for 2 weeks at 5° C., 6 of the 8 cloudy solutions were clear enough for ultraviolet absorption studies. The substances responsible for the turbidity gradually settled to the bottom of the tube.

Cholesterol Content. Cholesterol determinations were made on the 2 most turbid of the 0.01 N HCl solutions (Nos. 2 and 10) by the method of Schoenheimer and Sperry.[11] Neither of the preparations contained any detectable amount of cholesterol.

Phosphorus Content. Three globulin solutions, Nos. 4, 7 and 8, were mixed together and a phosphorus determination was carried out on the resulting mixture by the procedure of Fiske and Subbarow.[2] No phosphorus was detected.

Quantitative Protein Determinations. Colorimetric determinations of the proteins in the 0.01 N HCl solutions were made with the phenol reagent,[9] the cryoglobulin of the patient J. P., as reported in Paper I, being used as the standard.

The values for cryoglobulin, as thus obtained, are listed in Table 2.

Ultraviolet Absorption. Seven of the 0.01 N HCl solutions of cryoglobulins were sufficiently clear for ultraviolet absorption studies. The densities over the range 2400 to 3300 Å were determined with a Beckman quartz spectrophotometer with a hydrogen discharge tube and quartz cuvettes 1 cm. in thickness. Using the results of the quantitative determinations of these proteins, it was possible to calculate the ultraviolet extinction coefficients (Fig. 1). The extinction coefficients of the cryoglobulin from Samples 7, 8 and 9 were identical within experimental limits; so only the average curve was used to represent these proteins.

By comparing the ultraviolet absorption spectra of the cryoglobulin preparations with those of the normal plasma proteins[6] it is evident that the cryoglobulins differ from albumin. Five of the cryoglobulin preparations gave absorption curves similar to the gamma globulins and fibrinogen. Two of the preparations gave unusually high absorptions.

The absorption curve for the cryoglobulin from the case of familial hypoprothrombinemia (No. 3) is similar to that of a nucleoprotein.* A quantitative Bial's test for ribose was carried out on this

TABLE 2.—CASES WITH POSITIVE CRYOGLOBULIN, CEPHALIN-CHOLETSEROL, OR SERUM DILUTION TESTS

Case No.	Diagnosis	Grade of cryoglobulin precipitate	Cryoglobulin (mg. %) (serum)	Cephalin-cholesterol test (48 hrs.)	Serum dilution test (48 hrs.)
1	Cryoglobulinemia and cardiovascular renal disease	3	800	0	
2	Lymphatic leukemia	2	25	0	0
3	Congenital hypoprothrombinemia	2	6.9	2	0
4	Bronchopneumonia	2	7.6	0	0
5	Bronchiectasis	2	20	0	0
6	Rheumatic heart disease	2	12	0	0
7	" " "	2	12	0	0
8	Subacute bacterial endocarditis	2	21	0	0
9	" " "	2	19		
10	" " "	2	25	4	2
11	" " "	2	..	2	0
12	" " "	2	..	2	0
13	" " "	2	..	0	1
14	Rheumatic heart disease	1	Trace	0	0
15	" " "	1	Trace	0	0
15	Pneumonia	1	Trace	0	0
17	Pleural effusion	1	Trace	0	0
18	Empyema (tbc)	1	Trace	0	
19	Emphysema	1	Trace		
20	Cardiovascular renal disease	1	Trace	0	0
21	Brucellosis	1	Trace	0	0
22	Ulcerative colitis	1	Trace	0	
23	Embolic femoral artery occlusion	1	Trace	0	
24	Hepatic cirrhosis	1	Trace	4	0
25	Diabetes	1	Trace	0	
26	"	1	Trace	0	0
27	Addison's disease	1	Trace	0	0
28	Polycythemia vera	1	Trace	0	0
29	Idiopathic purpura	1	Trace	3	1
30	Carcinoma of pancreas	1	Trace	0	0
31	Lymphatic leukemia	1	Trace	0	2
32	Myeloid leukemia	0	0	3	0
33	Hodgkin's disease	0	0	3	0
34	Polycythemia vera	0	0	3	0
35	Carcinoma of pancreas	0	0	2	0
36	Multiple myeloma	0	0	0	2
37	" "	0	0	..	2

* C. P. Barnum (unpublished data).

preparation. Five per cent of the protein appeared to be ribose. This may indicate that the protein was a nucleoprotein, but only a very small amount of material was available, and the Bial's test may not have been specific for ribose. The electrophoretic pattern of the plasma proteins of this case was essentially normal.

approximating that of fibrinogen, it is likely that these proteins are not fibrinogen.[7] Since most of the spontaneously precipitated proteins have ultraviolet absorption curves similar to those of the gamma globulins, these proteins probably belong to the gamma globulin group. The cryo-

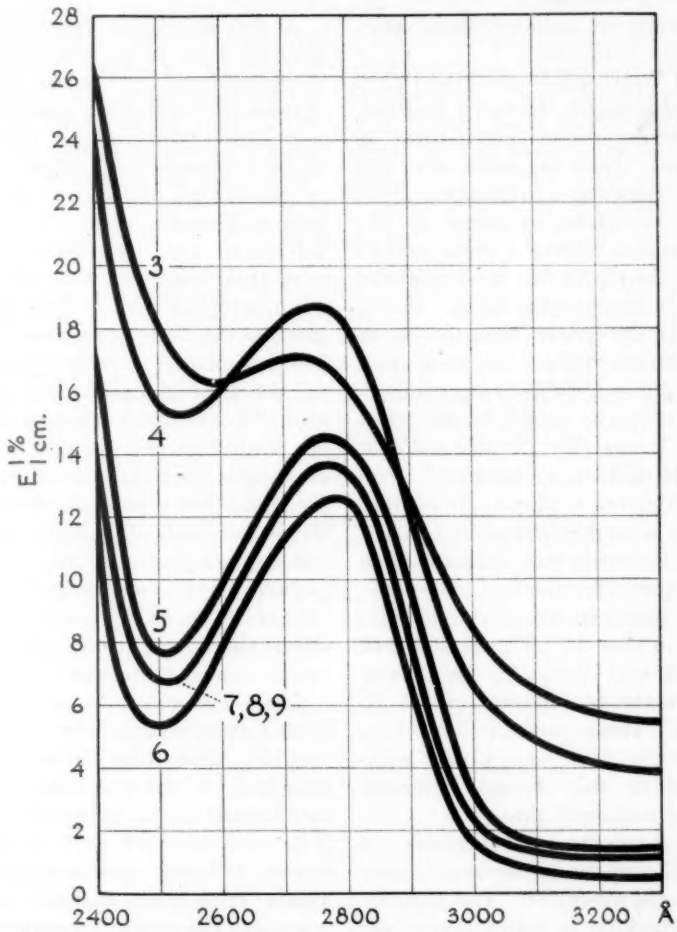

FIG. 1.—Ultraviolet extinction coefficients for cryoglobulins of Samples 3 to 9 inclusive. These were determined with a Beckman quartz spectrophotometer with a hydrogen discharge tube.

Discussion. The present results indicate that several different proteins may precipitate spontaneously from cooled abnormal serum. In view of the fact that none of the proteins from the cases discussed in Paper I had a molecular weight even

globulin isolated from the case of hypoprothrombinemia may be a nucleoprotein.

It is evident that cryoglobulins may be present in the serum of patients with a great variety of diseases, while normal serum does not appear to contain this

type of protein. The concentration of cryoglobulin in pathologic serum is quite variable. In some cases it is present in such large quantities that it may well relate to the patient's symptoms and findings as in the case reported in the preceding paper. Cryoglobulinemia may be considered a non-specific indication of a disease process comparable to fever, increased erythrocyte sedimentation velocity, and other symptoms.

Stein and Wertheimer[12] reported that 5 of 7 cases of subacute bacterial endocarditis exhibited protein precipitates in cooled serum. They suggested that this may be of diagnostic significance. However, since cryoglobulins occur in the serum of patients having a great variety of diseases, their presence is of doubtful significance in any specific sense. In the present study the serums from 6 cases of subacute bacterial endocarditis were studied. In all of these (Table 2) cryoglobulin was observed in the cooled serum. The diagnosis in 1 case (No. 13) was not definitely established, *i. e.*, positive blood cultures could not be obtained. In another case (No. 8) a marked reduction of cryoglobulin in the serum was apparent after 3 weeks of penicillin therapy. Visually, the smallest concentration of cryoglobulin was present in Case 11. This patient had been treated with penicillin for several weeks before the serum was studied for cryoglobulin. These results indicate that cryoglobulinemia is at least a very common finding in this disease, although probably not uniformly present.

There are 2 reports in the literature of patients with purpura hemorrhagica associated with cold allergy.[5,10] The histories and clinical findings in these 2 cases are quite similar to those of J. P. (see Paper 1). There are also reports of cases with sensitivity to cold with urticarial lesions and "red blotches."[13] It is unfortunate that no observations were made on the cooled serum from these patients. At present one can only speculate on the presence of cryoglobulinemia in these instances.

Coburn and Moore[1] have recently shown that there is an increase in serum gamma globulin in patients with lupus erythematosus. They also observed that in the descending limb of the electrophoresis cell, where the other proteins moved away from the gamma globulins, there was an opaque precipitate. This suggests the presence of a cryoglobulin in the serum of their cases.

Abnormally large amounts of gamma globulin occur in hyperimmune antipneumococcus horse serums and in the serums of cases of liver disease, multiple myeloma, sarcoidosis, lymphopathia venereum, and lupus erythematosus. Since most of the cryoglobulins probably belong to the gamma globulin group, it would be of interest to study the serums from patients with these diseases. One should bear in mind, however, that an increase in gamma globulin does not necessarily indicate the presence of cryoglobulin. This is shown by the lack of correlation between the cephalin-cholesterol flocculation test and the occurrence of cryoglobulin. In 1 case of hepatic cirrhosis, not included in the foregoing, the cephalin-cholesterol flocculation was markedly positive as was also the thymol turbidity test. The serum globulin in this case ranged between 8 to 10 gm. %, most of which was revealed electrophoretically to be gamma globulin; yet no cryoglobulin was present.

Lipid Turbidity. On several occasions fresh fasting serums were observed to be turbid. After these turbid serums were kept at 5° C. for 1 to 3 weeks, a precipitate formed on the surface as a white ring. This was observed in 7 serums from a variety of cases: glomerulonephritis, leukemia, pneumonia, diabetes and aplastic anemia. The substance appearing on the surface was assumed to be of lipid character. Its behavior was entirely different from the cryoglobulins.

Eleven diabetic serums were studied. These were collected in the fasting state. Only 1 of the freshly prepared serums was turbid, but after the serums were kept at 5° C. for 48 hours, 8 became turbid. Again, as these stood at 5° C. for longer

periods, a precipitate formed on the surface as a white ring, the serum below remaining clear.

Most serums are turbid when obtained shortly after a meal. Upon standing for 1 to 2 days the lipids in these serums rise to the surface.

Summary. 1. In a study of 121 serums from as many individuals, suffering from a variety of pathologic conditions, spontaneous precipitation of protein from the cooled serum was observed in 31 instances. The presence of these proteins, which are designated as cryoglobulins, may be regarded as a non-specific indication of a disease process. They were not encountered in any of the serums from 40 normal individuals.

2. Serums which contained cryoglobulins were divided arbitrarily into 3 grades on the basis of amounts. These are as follows:

Grade 1	From a trace to 6 mg. %
Grade 2	From 6 to 25 mg. %
Grade 3	Greater than 25 mg. %

Grade 3 cryoglobulinemia was encountered in but 1 case, and in this instance there is reason to believe that the cryoglobulin was related to a severe purpura which stood in the foreground of the clinical picture.

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HEPATIC CALCULI

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Hepatic calculi (hepato-lithiasis) have been rarely diagnosed clinically, are usually unsuspected, and found only at operation or necropsy. The literature, especially American, on this subject is sparse, yet Best[2] in an analysis of the literature states that in 456 cases of cholelithiasis there were 35 cases of liver stone. This represents an incidence of liver stones in cholelithiasis of 7.6%. Courvoisier (1903) in 16,025 autopsies found 1714 cases of cholelithiasis in which there occurred 1 case of liver stone. Hesse[7] (1911) in 17,402 autopsies had 278 cases of cholelithiasis and 1 case of liver stones. However, Miyake[8] (1913) in 257 cases of cholelithiasis had 20 cases of liver stones. Apparently in Japan, liver stones are relatively frequent. Hepatic calculi may also occur without cholelithiasis. In 53 cases of liver stones analyzed by Rufanov,[9] 20% had calculi confined to the liver alone, while 80% had calculi also present in the gall bladder, bile passages or both. The present paper briefly reviews the subject of hepatic calculi and describes 4 cases. It also discusses the possibility of diagnosis, and the clinical significance of this condition.

Classification of Hepatic Calculi. Liver stones may be grouped as follows:

1. Firmly fixed stones within the intrahepatic ducts. These may be single or multiple and the size of the calculi varies widely.
2. Isolated, circumscribed lesions in the liver with stones embedded in a cavity containing bile or pus.
3. A more diffuse process with the intrahepatic ducts containing small stones, gravel or débris.
4. Diffused, small abscesses containing stones or gravel and associated with a purulent cholangeitis.

Incidence. It has already been stated that liver stones have been found in as many as 7.6% of cases of cholelithiasis which came to necropsy. This incidence would seem to vary directly with the thoroughness with which all the bile ducts of the liver are examined. Hepatic calculi have been described in every decade from the 2nd to the 9th. In contrast to gall stones, there is no difference in rate of occurrence of hepatic calculi between the sexes.

Etiology and Pathogenesis. The etiology of hepatic calculi is not definitely known. The various theories advanced for the genesis of gall stones apply equally to liver stones. These may be summarized as infection, stasis, disturbed metabolism (especially that of cholesterol), abnormalities in the chemistry of bile, dysfunction of the autonomic nervous system, and, perhaps, changes in the hepatic cell membrane. The immediate mechanism causing the formation of stones is probably chemical,[3] due to variations in the ratio of concentration of bile acids, fatty acids, cholesterol, calcium and protein, together with changes in the pH of bile. Hepatic calculi are usually of the bilirubin-calcium type[13] but they may have variations similar to those found elsewhere in the biliary system.

Clinical Aspects. The symptoms vary widely from the symptomless to those showing evidence of biliary obstruction. Since the majority of patients have coexisting stones in the gall bladder and/or bile ducts, it is difficult to describe characteristic symptoms of hepatolithiasis. The most frequent symptom is pain, usually epigastric or in the right upper quadrant, occasionally radiating to the right scapula. This pain occurs in attacks which may be of long duration. Fever is the next most

common finding, and in 25% of cases there are chills. These, when they occur, are due to cholangeitis, liver abscesses or pleurisy. The temperatures may be normal in uncomplicated hepatolithiasis. Jaundice is frequently encountered, is of the obstructive type, and may be accompanied by pruritus, hemorrhage and changes in the urine and stool. Gastric disturbances are frequent, indigestion, anorexia and vomiting during attacks are the rule. Very often inanition and anorexia are the earliest symptoms of hepatic calculi. With stones also present in the biliary tract, the symptoms would be more inclined to be those of cholecystitis or obstruction of the biliary passages, the stones in the liver causing no symptoms except those of a general nature (inanition, anemia, fatigue, etc.). The liver, or one of its lobes, is usually enlarged, but it may be normal in size. There may or may not be tenderness and spasm on palpation of the abdominal wall. Achylia is common. In secondary involvement of other organs we find chronic, secondary, indurative pancreatitis, which seems to be less than that found in cholecystitis. A right-sided pleural involvement with serous or purulent exudate may occur, and damaged kidneys have been reported.

Diagnosis and Differential Diagnosis. It is readily noted that there is no pathognomonic symptom complex for this condition. Clinically, the diagnosis of hepatic calculi is at best difficult, if not impossible without the Roentgen ray. There may be a complete absence of symptoms. With symptoms, the differential diagnosis includes cholecystitis, cholelithiasis, obstruction of the common duct, cholangeitis, liver abscess and other diseases of the gall bladder and bile ducts.

The roentgenogram affords a much more accurate means of diagnosis. Walters and Snell[13] suggest that small irregular opacities occasionally seen in the hepatic area on Roentgen ray may be due to intraductal stones. Volpe[12] described a solitary hepatic calculus as a mulberry-like shadow. The hepatic calculi in our Case 1 are almost uniformly spherical in appearance, varying in diameter from 0.5 to 2 cm. There is an outer shell of rather dense calcification. The core is much less opaque and is studded with round areas of radiolucency. These are probably due to an irregularity in the deposition of calcium, but there is a possibility that the calculi are canalized by bile.

Other causes of calcification in the liver must be considered in the differential diagnosis. These include: (1) Hemangiomas: hemangiomas of the liver are almost always of the cavernous type. When they calcify they exhibit numerous trabeculations arising and radiating from a common center.[1]

2. Amebic abscess: calcification occasionally occurs in chronic abscesses. There is almost always a history of amebic infection and the calcification is in the form of dense irregular shadows.[10]

3. Echinococcus cyst: in chronic cysts, calcification may occur in the capsule. The calcification is in the form of concentric strips alternating with transparent strips.[4]

4. Gummata: gummata usually do not calcify. When calcification does occur, it is usually trabecular in nature.[5] There is evidence of lues elsewhere.

5. Tuberculomas: there is almost always evidence of tuberculosis elsewhere, including calcified tuberculomas of the spleen. They appear as spheres with a central density having a surrounding moat.[5,11]

6. Carcinoma: Hamburger[6] described a calcified primary carcinoma of the liver in a 5 year old child. The calcification was attributed to irradiation of the tumor.

7. Hydatid cysts, calcified subphrenic abscesses, calcified infective granulomata, calcified infarcts and metastases should be readily differentiated from hepatic calculi by the character of their appearance, their location and the clinical picture.

Treatment and Prognosis. The treatment of hepatic calculi will vary greatly with the individual patient. In those cases with no symptoms, or with minimal

symptoms and no stones in the extrahepatic biliary system conservative treatment is indicated. This would include a low fat, high carbohydrate, high protein diet with adequate vitamin intake. Operative treatment depends in great degree on associated findings such as gall stones, common duct stones, etc. Rufanov[9] in analyzing 55 cases states that the most frequently performed operations were cholecystectomy, choledochotomy, hepaticotomy and cholecystostomy. According to Rufanov[9] the overall mortality of cases undergoing surgery is 60 to 65%. The best prognosis is in the younger age group (under 50 years) who have had a relatively short duration of illness (under 3 years). In these cases the pathologic process is usually limited in extent and there is no general damage or insufficiency of the liver parenchyma.

The highest operative mortality occurs in people over 50 years of age. Usually the disease is of long duration and there are severe pathologic changes in the liver and other organs (kidney, pancreas, etc.). Ninety per cent of these cases have coëxisting stones in the gall bladder and at autopsy all the bile ducts to their most minute branches are filled with compactly packed stones.

Another group may be mentioned, namely elderly people who have had prolonged symptoms of cholelithiasis with or without duct obstruction and have not undergone surgery. These are usually seen shortly before autopsy and a severe cholangeitis, liver or subdiaphragmatic abscess, ulceration of the gall bladder or bile ducts, cholemia, hemorrhage, sepsis and collapse are encountered.

The principal causes of postoperative death are hepatic insufficiency or a purulent process, *e. g.*, cholangeitis, liver abscess. Second laparotomies are greater in number in hepatolithiasis than in cholelithiasis. This is due to the severity of the liver stone pathology, to overlooking the presence of liver stones at the time of original operation, and to short periods of drainage of the ducts.

Case Report. CASE 1. (Referred by Dr. Atwood, Boston, Mass.) A married woman of 62, mother of 2 children, has been under Dr. B's care for 29 years. When first seen in 1917 when she was 33 years of age) she gave the history of appendectomy for an acute appendicitis 7 years before and removal of a cervical rib when 2 years of age. She stated that "she had not felt well since her last child was born, and was suffering from constipation, poor endurance, fainting spells and occasional vomiting." She was allergic to fish, complained of intestinal gas, passing mucus from rectum, and dizzy spells with attacks of cardiac palpitation. She weighed 89 pounds; a reduction of the best weight she ever had of 11 pounds; her blood pressure was 117/88. The diagnosis made at that time was viceroptosis, ileocolonic stasis, neurosis and general debility. As a result of bed rest, abdominal support, and medications she improved and reached a weight of 117 pounds in a few months time.

She was referred back to Dr. Atwood, and living some distance from New York, was seen at about yearly intervals without anything of moment occurring. In 1931 the menopause began, with the usual vasomotor symptoms which were controlled by ovarian extracts. About that time she developed anemia, the red cells dropping as low as 2,700,000 per c.mm. This was controlled by hematinics and diet.

Up to 1939 the diagnoses made and treatments employed were for indigestion, slight anemia, debility, periods of exhaustion, vague abdominal pains, arthritic pains due to a slight degree of arthritis of the atrophic type, constipation and insomnia.

In 1939, after about 2 years of complaints of more fatigue and indigestion, she was reexamined thoroughly. There were 2 abscessed teeth, there was a moderate fusiform dilatation of the aorta. The blood pressure was 150/86. The liver was not enlarged or tender. The Wassermann was negative. Laboratory findings included: blood sugar, 109 mg.; non-protein nitrogen, 33 mg.; calcium, 11.3 mg. (top normal); phosphorus, 3.5 mg.; cholesterol, 280 mg. (normal 125 to 210); uric acid, 3.2 mg.; and total protein, 8.4 gm. per 100 cc. A slight hypochromic anemia was present. The stomach was achylic even to histamine and Dr. B's pancreatic test was half normal in amylase units. The liver function tests were normal

(bromsulphalein, cephalin flocculation, hippuric acid) as were also the urine and feces. The Roentgen rays were not particularly significant with the exception of the liver, which showed a number of calculi scattered throughout the organ. Several Roentgen ray examinations of the liver were made since 1939 showing continuously about the same condition throughout the years.

CASE 2. A 77 year old white female was first admitted to St. Vincent's Hospital, New York City, in August 1938, because of dyspnea and epigastric pain of 1 day's duration. Except for an operation 40 years previously because of an ovarian cyst, she had always enjoyed good health. There was no history of jaundice or biliary colic. Physical examination revealed an elderly, thin female in moderate respiratory distress. The positive physical findings were limited to the heart and lungs. The heart was enlarged, especially the left ventricle, as was the aorta. The rhythm was irregular and the blood pressure was 170/90. There were fine moist râles at both lung bases. The abdomen was negative and the liver not

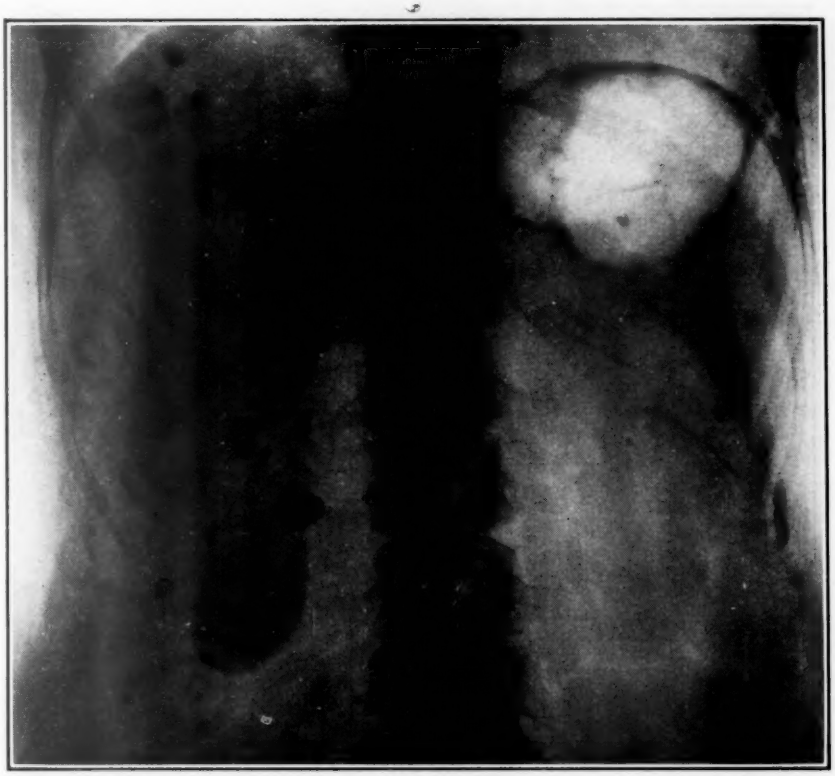

FIG. 1.—Film taken in 1939 showing liver stones. Gall bladder normal.

Comment. Here was a physically unendowed woman with a series of minor complaints over years of time. The time of onset of the liver stones is not known. It is possible that the anemia or exhaustion was in some way bound up with their advent. The fact that stones may occur in the liver and there be none in the gall bladder or ducts suggests that an abnormal metabolic process may occur in the liver cells. The high content of blood calcium and cholesterol seems significant. She never had any definite symptoms pertaining to the liver and is today about in the same reasonable state of health as she has been for years.

enlarged. The urine showed 3+ albumin, 30 leukocytes per high power field and a specific gravity of 1.015. The blood count showed 4,400,000 red cells, 14 Gm. hemoglobin, 10,200 leukocytes, with 88% neutrophils, 11% lymphocytes and 1% eosinophils. Blood non-protein nitrogen and sugar were 48 mg. and 82 mg., respectively, per 100 cc. and the Kahn reaction was negative.

bases. Her heart was unchanged from the first admission. The liver was enlarged 5 finger breadths below the costal margin, was smooth and slightly tender. The blood count showed 3,300,000 red cells, 70% hemoglobin, 19,200 leukocytes with 76% neutrophils. The non-protein nitrogen was 32 mg. and the icteric index 32. For the 1st week, the temperature ranged between 100° and

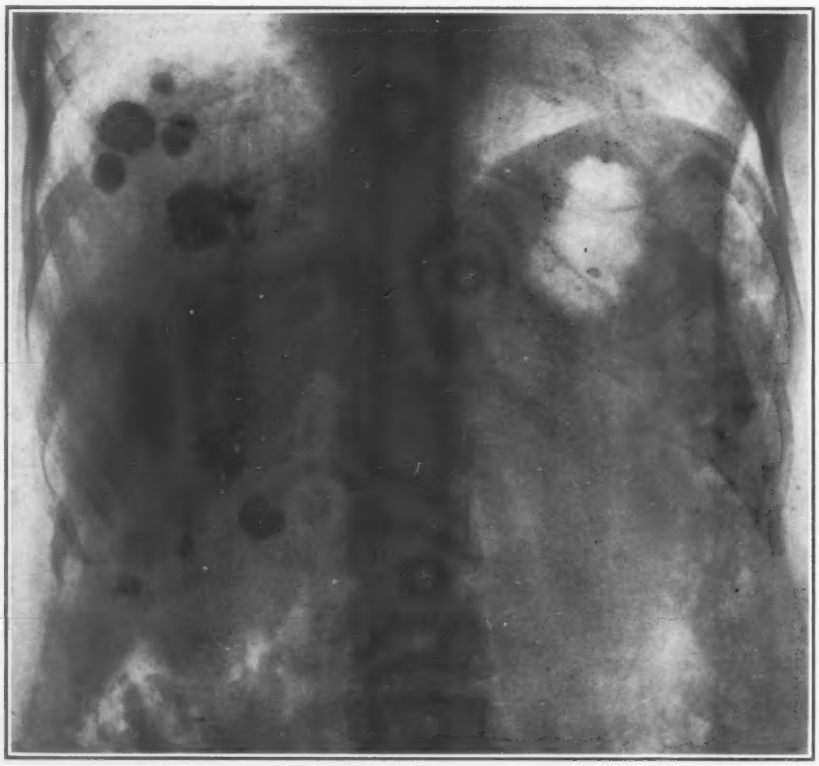

FIG. 2.—Postero-anterior film taken in 1946 showing the same as above. Gall bladder films taken separately still show a normal gall bladder.

She was digitalized and rapidly improved and was discharged from the hospital in 1 month.

The second admission was in May 1939, following severe pain in the left posterior chest with slight fever of 5 days duration. She had been dyspneic for the preceding 4 months. At the time of admission she was acutely ill, dyspneic, orthopneic and the skin was jaundiced. The positive physical findings were moderate dependent edema, dullness and diminished breath sounds in the left lower lobe and râles at both lung

102° F. She gradually became compensated on digitalis and diuretics and the jaundice disappeared. She was discharged 1½ months after admission.

The last admission was in February 1940, when she suffered a cerebral vascular accident. She was comatose on admission and died shortly thereafter at the age of 79.

At autopsy, cerebral atherosclerosis and encephalomalacia and hemorrhage in the left parietal and frontal lobes were found to be the immediate cause of death. The heart was hypertrophied and dilated. There was

FIG. 3.—Right lateral film taken in 1946.

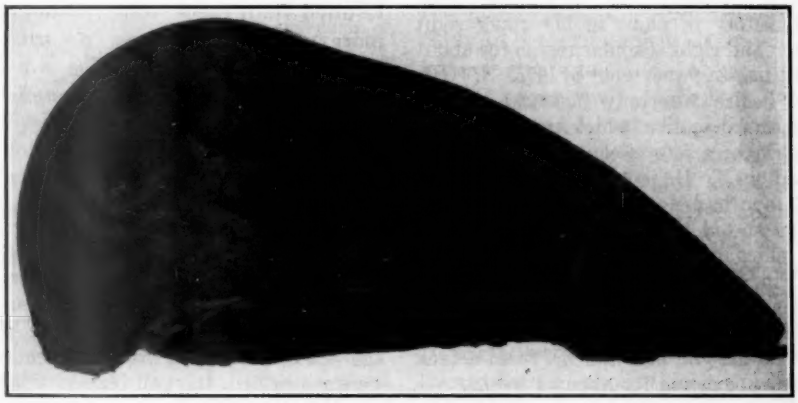

FIG. 4.—Liver stones in large dilated areas of the bile duct. Small granules of stone scattered over the cut surface of the liver.

acute verrucous endocarditis of the aortic cusps. There was a lobular pneumonia of the lungs. The liver (Fig. 4) was not enlarged, weighing 1100 gm. The surface was smooth and on section was yellow-brown in color. The bile ducts in the right and left lobes close to the hilus were dilated and filled with faceted, pigmented stones. Microscopically the liver lobules were atrophied, especially in the central portions.

The gall bladder was filled with pigmented faceted stones. The common duct and both hepatic ducts were dilated and contained the same faceted stones. There was apparently no obstruction to the flow of bile.

Comment. In this familiar type of cardiac case it is probable that at the second admission the icterus may have come from a pulmonary embolism and not from the calcareous condition in the liver and biliary tract. In the second admission her liver was definitely enlarged, probably due to heart failure. The death was due to an apoplexy. From the first to the third admission there were no definite clinical signs of either liver or gall bladder stones and the liver was not enlarged at the last admission, as it had been at the second, 9 months before. Considering the history before her first admission, she was in "good health" even though she must have had both liver and biliary tract stones for years.

Case 3. A dentist, 42 years of age, with a past history of pains in his upper right abdomen and right scapular region for about 6 years before, was seen by Dr. B. His general health was only fair and he had periods of exhaustion which caused him to stop work for a few weeks until he became "bucked up." He continued at his work until 4 months before first seen by Dr. B., when he stated that as a "result of a fire in his office he became jaundiced." This cleared in about 3 weeks time. However, he had an attack of jaundice 1 year before following an operation for rectal fistula, and when seen first he had had his third attack of jaundice, existing for about 4 weeks.

Briefly, he was a jaundiced individual in a fair state of nutrition but showed a rapid sedimentation rate, a diphasic temperature and attacks of chills. A leukocytosis (19,000) and high neutrophil count (84%) suggested an infection. The Roentgen rays showed about 11 small stones scattered through his liver, mostly in the lower portion of the organ. These seemed to be solid stones high in calcium. He became ill quickly and was hospitalized, treated medically with the diagnosis of a possible septic cholangeitis. He made a symptomatic recovery in 4 weeks and went back to work in his office. About 3 weeks later he began complaining again, became moderately jaundiced and septic, and was operated upon by Dr. John F. Erdman who removed his gall bladder, appendix and established tube drainage. The tissue diagnoses were chronic cholecystitis without gall stones, acute periportal hepatitis, miliary abscesses of the liver and chronic appendicitis. The man recovered slowly, the wound healed perfectly and he went to a rest home. About 7 months after the operation there developed small growths along the upper half of the scar which were found to be "metastatic adenocarcinoma of the skin." At the time of the operation there was no evidence in his abdomen of malignant disease.

Comment. It seems reasonable to assume in this case that the liver stones had existed for years and, other than the possibility that the periods of exhaustion were due to them, gave no symptoms. The Roentgen ray evidence of hepatic calculi and findings at operation suggest that the abscesses of the liver may have resulted from these stones, although it is more probable that they occurred from the septic cholangeitis. The site of the carcinoma from which he succumbed was not determined.

Case 4. In 1931 a migrainous woman, 52 years of age, with 3 children, was treated by Dr. B. for a biotoxic intestinal condition. Her past history revealed that at the age of 25, after the birth of a child, she began complaining of gaseous indigestion and pain in the upper right abdomen. At 38 years of age cholecystectomy and appendectomy were performed, the gall bladder containing many stones. She had a reasonable degree of relief for 4 years, when gradually attacks of gas, anorexia, constipation and loss of weight returned. These were her complaints

when first seen. At subsequent examinations, after Roentgen ray, a diagnosis was made of liver stones. They were small in size and scattered throughout the liver like birdshot.

About 1 year later she became jaundiced. Examination of the biliary tract after the ingestion of dye revealed the extrahepatic ducts to be dilated and stones were outlined. Under conservative treatment the jaundice cleared but she never regained her former state of health.

At 63 years of age (1942) her health failed perceptibly. At times the liver was enlarged and tender. She ran a low grade fever from time to time and had recurrent mild attacks of jaundice. The icteric index was always above normal. The highest hippuric acid in 4 hours was found to be 4.7 gm. and the galactose tolerance in 5 hours was 0.3 mg.

For a period of months there was a marked abatement of symptoms, the liver function tests were normal, but the icteric index remained high. Her complaints gradually returned and, finally, after 2 weeks of preoperative preparation, she was operated upon by Dr. Raymond P. Sullivan in 1943 at the age of 64. At operation innumerable small stones of varying sizes were removed from the extrahepatic biliary ducts. They choked the ducts practically to the under surface of the liver. After removal of the T tube several more stones came out. The stones were calcium-bilirubin in character. No stones could be palpated in the liver substance at operation. She has greatly improved symptomatically in the past 3½ years. Roentgen ray still reveals the presence of stones in the liver.

Comment. It may be concluded from her physical and symptomatic improvement following operation, that her symptoms were due to the stones in the biliary tract and not to those in the liver. The icteric index is now at the upper limit of normal. The stones still remaining in the liver seem to produce no symptoms, although a mild degree of anemia and slight fatigue still persist.

Discussion. In Case 1 (uncomplicated hepatic calculi) the liver was not enlarged or tender and there were no symptoms suggesting the presence of hepatic stones. The only symptoms were general, suggesting a vague type of indigestion which could not be distinguished from those present in achylia or a low-grade pancreatitis. This case is also of interest because of the absence of stones in the extrahepatic ducts and gall bladder. Case 3 also had no extrahepatic stones, although the gall bladder was infected. The other 2 cases had both intra- and extrahepatic stones.

Hepatic insufficiency did not occur in Case 1 or 2 and both cases gave no specific symptoms relative to the biliary system. (It is assumed that the transient jaundice in Case 2 was due to a pulmonary infarct.) Both Case 3 and 4 had symptoms, 1 due to infection and the other to gall duct obstruction and mild infection. Thus we may say that in uncomplicated hepatic calculi the symptoms are minimal or absent. When symptoms occur, they are due to infection. In hepatic calculi complicated with extrahepatic biliary calculi, symptoms referable to the biliary system are more likely to occur and are indistinguishable from those produced by disease of the gall bladder or common duct in which no liver stones are present.

The only positive method of diagnosis is by roentgenogram, and unless the liver stones are calcified or of considerable size, they will not be discovered. In view of the statistics quoted earlier in this paper, it appears that a painstaking search of the intrahepatic ducts at autopsy, especially in cases of cholelithiasis, will reveal a much greater incidence of hepatic calculi than is at present thought to exist.

True uncomplicated hepatic calculi appear to be compatible with long life and comparative good health and the treatment should be conservative and supportive. It is reasonable to assume, if there is but a single hepatic stone or a cluster of stones in a small area, that their removal by operation should be considered. In the presence of complications such as liver abscess, cholelithiasis, cholangeitis and obstruction of the common duct, the treatment should be predicated on the complication and the presence of the liver stones be considered as of secondary im-

portance. However, it must be remembered that in the presence of liver stones the mortality rate in operations of the biliary tract is increased.

Summary. The incidence of hepatic calculi associated with cholelithiasis is probably about 6% when careful studies of the intrahepatic ducts are made at autopsy. The incidence is about equal for male and female. Hepatic calculi produce no characteristic symptoms. Usually symptoms are caused by associated stones in the extrahepatic biliary system or by complications such as infection. The diagnosis can only be made by roentgenogram.

Of 4 cases of hepatic calculi here presented, 2 were symptomless, although 1 of these also had stones in the extrahepatic biliary system. One case was complicated by infection (multiple small liver abscesses) and 1 case by obstruction of the common duct which was relieved by operation, and the stones remaining in the liver have now been asymptomatic for several years.

In true hepatic calculi with no complications, treatment is conservative and the prognosis is good. With complications such as obstruction or infection, surgical treatment should be directed toward the complication, in which case the mortality is greater than if no hepatic calculi are present. The principal causes of postoperative death are hepatic insufficiency or a purulent process, and as cholangeitis and liver abscess.

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STUDIES OF HEPATIC FUNCTION IN PULMONARY TUBERCULOSIS

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Chronic pulmonary tuberculosis although a localized disease will cause changes in other organs of the body by metastatic bacterial or toxic action. Pathologic changes in the liver are found not infrequently on postmortem examination. Together with chronic alcoholism pulmonary tuberculosis is the most frequent cause of fatty infiltration of the liver. In contrast to these findings there is no direct clinical evidence of significant liver damage in patients with chronic pulmonary tuberculosis. This apparent discrepancy stimulated the interest in functional liver studies in pulmonary tuberculosis and during the last 25 years a number of investigations were published concomitant with the development and perfection of liver function tests.

A review of these studies reveals that aagin in contradiction to the clinical observation considerable laboratory evidence of liver function disturbance was found. Lichtman[5] in his classical book on diseases of the liver states therefore, "while clinical evidence points to a lack of significant hepatic parenchymal damage in patients with pulmonary tuberculosis, sensitive liver function tests indicate the opposite." A critical study of these publications shows however that the function tests used were mostly non-specific and partly obsolete. This applies to the first publications in this field which appeared in the French literature,[1] as well as to the series of studies by Steidl and Heise,[8a,b] in this country. These authors found pathologic readings in most cases of far-advanced tuberculosis and in a later study almost identical results in patients with moderate and minimal involvement. It is interesting to note that whereas they found positive results with non-specific tests, *f.i.*, the Congo red test or since then discarded procedures such as the oxidation test, the bromsulphalein test as used in its original modification did not give any pathologic readings.

In a subsequent study by 2 other authors[4] the same test, 2 mg. dose, showed however some evidence of hepatic dysfunction. In 1942 by using the more sensitive 5 mg. per kg. test, Kruger and Gerber[3] found evidence of impaired function, especially in far-advanced protracted disease. They examined also a series of 20 patients with secondary amyloid disease and found significant retention of bromsulphalein in 18 patients. In contrast to their findings a previous study[9], which was entirely limited to patients with pulmonary tuberculosis and complicating amyloid disease, showed dye retention only in 2 out of 12 patients. These authors used however the original 2 mg. dose procedure.

The 3 last-mentioned studies showing negative results with the 2 mg. dose of bromsulphalein even in the presence of marked pathologic anatomic changes as in complicating amyloidosis, and demonstrating definite disturbance with the more sensitive 5 mg. dose modification, seem to point the way to a clarification of the problem. We felt that with the further improvement of the test in the form of the serial bromsulphalein test and also the other fairly specific tests as the intravenous hippuric acid test, the cholesterol ester determination, the cephalin cholesterol flocculation and the determination of a series of chemical serum compo-

nents related to liver function, a satisfactory result could be obtained. Based on the symposium on liver function tests[6] and the recent work on liver impairment in acute disease,[7] we set up a study of liver function in a group of far-advanced cases, utilizing the following tests and laboratory procedures.

Method. At first it was decided to select patients falling into the minimal, moderate, and far-advanced stages of tuberculosis. Later this was changed to include only a group of patients with far-advanced disease and without complicating tuberculous empyema or amyloidosis. All patients included in this series were carefully interviewed for previous history of jaundice or biliary colic. In addition, an excretory cholecystogram was done to rule out stones, and delayed emptying time of the gall bladder dye. Priodax was used with the standard technique advised.

Program. 1st day. 7 A.M. Patient urinates and discards specimen. All urine for succeeding 24 hours is collected in a brown bottle supplied by laboratory. This bottle contains 5 gm. of Na_2CO_3 and 100 cc. of petroleum ether. Bottle must be kept out of intense light. (Urobilinogen.) 7:15 A.M. 15 cc. of blood drawn for cephalin cholesterol, bilirubin, cholesterol (total and ester) and phosphatase.

2nd day. 7 A.M. Patient urinates to complete 24 hour specimen. 7:15 A.M. 13 cc. of blood drawn for NPN, albumin, globulin and fibrinogen. 7:30 A.M. Serial bromsulphalein test.

3rd day. 7:30 A.M. Intravenous hippuric acid test.

Results. In Table 1, attention is called to the fact that the non-protein nitrogen was within normal limits in every instance. Similarly, the total serum proteins were all in the upper levels of normal with normal albumin, globulin and fibrinogen amounts and ratios. While these figures cannot be used as a true index of nutritional status, they at least show that the nitrogen levels were maintained.

In regard to bilirubin, normal values for circulating bilirubin vary within wide limits. According to Lichtman,[5] values up to 0.5 mg. % have been found in normal healthy humans, while Cantarow and Trumper[2] quote up to 0.8 mg. as the high norm, with 75% of cases being below 0.5 mg. In this small series, figures ran from 0.13 to 0.60 mg., none high enough to be impressive. As for urobilinogen, normal values of 1 to 4 mg. are usually eliminated daily in the urine and our figures varied from 0.09 to 2.76 mg., again within normal limits.

In Table 2, figures are given for the same 12 patients for studies in cholesterol as total free esters, and free as calculated for % of total. Normal total cholesterol figures range from 150 to 250,[5] although another authority[2] places the lower normal as slightly less (140). The ratio of free cholesterol to the total ranges in the normal from 20 to 40%. Two patients, Nos. 7 and 12, showed significantly lower total figures, 111.8 mg. and 104.6. Both, however, showed normal ratios of esters and free cholesterol and in neither case was the ester figure below the "critical level" of 60 mg. Parenchymal liver damage is usually associated with a decline in the total cholesterol as well as the ester fraction but the decrease in the latter is relatively greater than in the total. Factors other than liver function disturbances may influence the total cholesterol and its fractions, and it is considered unwise to regard this as more than an "omen" of liver damage according to Lichtman.[5]

There is considerable difference of opinion as to the value of prothrombin index as test of hepatic dysfunction. Normal and above normal findings may be misleading and even subnormal values cannot be considered as evidence of true dysfunction. If values of 90 to 100% are regarded as normal, Cases 3, 7, 8, 9 and 12 were below 89% and should be regarded as subnormal. The influence of absorption of vitamin K from the gastro-intestinal tract upon the prothrombin index cannot be measured except by the injection of this substance. Whether the change in prothrombin time following such injection can be taken as an index of hepatic function is still a moot question.

Increased serum alkaline phosphatase activity has been noted in patients with active tuberculosis.[2] While not truly a test of hepatic function, it is almost invariably increased in clinical and experimental forms of hepatocellular jaundice. Bodansky uses a high norm of 4 units while Cantarow and Trumper[2] insist upon a considerably higher value, up to 11 units, is also of interest that while 12 of the 18 cases showed values greater than 4 units, only 2 were above the higher norm of 11 units, and only 1 with a significant increase, readily explained.

Quick's modified intravenous hippuric acid synthesis, considered an important test of liver function is apparently little affected in tuberculous patients. Accord-

TABLE 1.—BLOOD CHEMISTRY IN CASES OF ADVANCED TUBERCULOSIS

				Serum protein			
	Bilirubin (mg./100 cc.)	Urobilinogen (mg./24 hrs.)	Non-protein nitrogen (mg./100 cc.)	Total (gm./100 cc.)	Albumin (gm./100 cc.)	Globulin (gm./100 cc.)	Fibrinogen (gm./100 cc.)
1. B. H.	0.44	0.24	30	7.78	4.60	2.79	0.39
2. H. B.	0.53	0.26	29	7.94	4.91	2.87	0.16
3. J. D.	0.30	0.67	33	7.54	4.29	2.98	0.27
4. L. Y.	0.35	0.15	30	7.37	4.08	2.60	0.69
5. G. T.	0.43	0.09	26	8.02	4.49	2.99	0.54
6. W. H.	0.32	0.05	30	7.78	4.78	2.54	0.46
7. J. M.	0.60	0.21	25	8.13	4.32	3.31	0.50
8. W. D.	0.34	0.25	26	7.21	3.81	2.77	0.63
9. A. K.	0.59	0.13	28	7.80	4.87	2.30	0.63
10. I. K.	0.24	0.18	19	8.33	4.00	3.52	0.81
11. G. H.	0.51	2.76	30	7.82	5.16	2.03	0.63
12. M. L.	0.13	1.60	27	8.12	3.66	3.65	0.81

TABLE 2.—HEPATIC FUNCTIONAL TESTS IN CASES OF ADVANCED TUBERCULOSIS

					Cholesterol			
	Cephalin cholesterol	Hippuric acid	Prothrombin index calculated as % of normal	Alkaline phosphatase (Bodansky units)	Total (mg./100 cc.)	Free (mg./100 cc.)	Esters (mg./100 cc.)	Free as % of total
1. B. H.	Neg.	1.00	90.0	5.30	228.2	76.4	151.8	33.5
2. H. B.	Neg.	0.46	86.3	5.41	158.8	34.6	124.2	21.8
3. J. D.	Neg.	1.11	80.9	5.20	144.5	48.6	95.9	33.6
4. L. Y.	Neg.	1.13	88.9	6.50	236.0	74.9	161.1	31.7
5. G. T.	Neg.	1.16	112.4	2.50	136.3	41.6	94.7	30.5
6. W. H.	Neg.	1.34	89.0	4.30	143.2	38.9	104.3	27.2
7. J. M.	Neg.	1.22	86.9	6.80	111.8	37.2	74.6	33.3
8. W. D.	Neg.	1.70	85.2	12.90	150.0	57.4	92.6	38.3
9. A. K.	Neg.	1.46	76.5	7.30	197.3	62.0	135.3	31.4
10. I. K.	Neg.	1.40	118.7	6.10	189.7	54.1	135.6	28.5
11. G. H.	Neg.	0.86	90.8	28.80	209.0	62.0	147.0	29.7
12. M. L.	4+	1.09	75.0	2.50	104.6	36.4	68.2	34.8
13. G. W.	..	..	..	6.10				
14. B. W.	..	..	..	3.10				
15. M. D.	..	..	..	3.20				
16. F. B.	..	..	..	2.40				
17. N. E.	..	..	..	3.70				
18. E. K.	..	..	..	4.40				

before placing any significance in the test. There was only 1 significantly increased value in the series of 18 cases, Patient 11, with a figure of 28.8 units (Bodansky). It is interesting to note that this patient has both a far-advanced pulmonary lesion as well as progressive osseous involvement. This latter destructive process could very well explain the high values obtained. It

ing to Quick, any urinary output of less than 0.7 gm. of benzoic acid as hippuric acid in the 1 hour urine specimen indicates some degree of impairment of hepatic function. In only 1 case (No. 2) was the reported value subnormal, 0.46 gm. The same negative results were found in Hanger's cephalin cholesterol flocculation test using Difco unripened cephalin as

advised by Mateer *et al.*[6] Case 12 showed a 4+ reaction while all the others were negative.

Of all the studies done in this series, the bromsulphalein test is the most important in the diagnosis of disturbance of the excretory function of the liver. By throwing a large excretory load upon the liver, marginal impairment of function may be detected. Diffuse disease of the liver without obstruction supposedly is best directed with this method. The modified appearance of the dye after injection of 5 mg. of bromsulphalein per kg."

Table 3 shows the results of the 5 mg. test in 17 patients using the 45 minute period. Seven cases, Nos. 2, 6, 7, 8, 10, 11, 12 and 15, showed retention of the dye.

Discussion. In the study of hepatic function, the choice of tests is difficult because of the large number as well as the many conflicting claims made for each. It has been demonstrated by many workers that there is no constant correla-

TABLE 3.—SERIAL BROMSULPHALEIN TEST IN FAR-ADVANCED TUBERCULOSIS

	% retention measured at 5 minute intervals after injection								
	5	10	15	20	25	30	35	40	45
1. B. H.	85	20	8	4	QNS	2	0	0	0
2. H. B.	60	30	20	15	10	7	7	7	5
3. J. D.	70	35	15	7	4	0	0	0	0
4. L. Y.	65	25	15	7	6	5	0	0	0
5. G. T.	65	25	15	7	2	12	0	0	0
6. W. H.	80	35	20	10	8	7	6	6	4
7. J. M.	85	25	15	10	8	6	5	4	2
8. W. D.	70	40	20	15	12	10	8	8	7
9. A. K.	90	35	20	10	7	0	0	0	0
10. I. K.	60	20	10	5	2	0	0	0	0
11. G. H.	90	40	30	20	20	QNS	QNS	20	20
12. M. L.	..	..	..	..	..	..	7	..	7
13. G. W.	55	40	25	20	20	18	18	18	18
14. B. W.	55	20	10	5	3	0	0	0	0
15. M. D.	55	25	15	7	5	0	0	0	0
16. N. E.	90	55	35	20	15	10	7	5	4
17. E. K.	45	22	12	7	6	4	0	0	0

TABLE 4.—A STUDY OF ABNORMAL VALUES IN HEPATIC FUNCTION

	Hippuric acid	Cephalin cholesterol	Phosphatase	Cholesterol		Bromsulphalein 45 min.)
				Total	Esters	
2. H. B.	0.46	Neg.	5.41	158.8	124.2	5
6. W. H.	1.34	Neg.	4.30	143.2	104.3	4
7. J. M.	1.22	Neg.	6.80	111.8	74.6	2
8. W. D.	1.70	Neg.	12.9	150.0	92.6	7
11. G. H.	0.86	Neg.	28.8	209.0	147.0	20
12. M. L.	1.09	4+	2.5	104.6	68.2	7
13. G. W.	..	..	6.1	..	..	18
16. N. E.	..	..	3.7	..	..	4

test using 5 mg. per kg. dose was applied taking 5 minute serial samples of blood up to 45 minutes. Mateer *et al.*[6] have carefully studied the entire problem of normal and abnormal results and definitely state that in a study of normal young people, "the dye had completely disappeared from controls in 30 minutes, in 86% in 35 minutes, in 96% in 40 minutes and in 100% . . . in 45 minutes. The 45 minute period has been adopted therefore as the normal standard for complete dis-

tion between results obtained from different tests in the same individual. The specific objections levied against function studies may be stated as (1) the tremendous reserve power of the liver; (2) the numerous functions of the liver so that any one test is insufficient to judge the function of the whole; and (3) the tests lack sensitivity.[5] These objections have all been answered by Lichtman who also states "It has long been recognized that

there is no correlation between structure and function of the liver cells."

Although the actual pathologic process in the liver of tuberculous patients usually is not a striking one, one may expect to find functional abnormalities in such patients with a toxic wasting illness affecting all of the body so profoundly. It is therefore not unexpected that patients with far-advanced long-standing tuberculosis would show changes in one or another of the functional studies done.

A recapitulation of the abnormal findings in Table 4 show that the only patient with the best correlation of abnormal function was M. L. (No. 12) with a 4+ cephalin cholesterol flocculation test, a low cholesterol and retention of bromsulphalein. This patient had extensive amyloidosis with a 100% retention of Congo red, an enlarged liver and spleen, and albuminuria. The only other amyloidosis patient in this series, G. H. (No. 11), showed a high phosphatase activity explained possibly by extensive osseous tuberculosis, and a considerable bromsulphalein retention, but with otherwise normal findings.

A careful study of Table 4 shows that there is no uniform correlation of findings, with hippuric acid synthesis and cephalin cholesterol flocculation showing the least positive, while the bromsulphalein excretion gave the greatest number of positive results.

Conclusions. 1. Hepatic function studies were done in cases of far-advanced pulmonary tuberculosis.

2. In addition to static values for NPN, serum proteins, total cholesterol and cholesterol partition, prothrombin phosphatase bilirubin, urobilinogen, other studies were done such as Hanger's cephalin cholesterol flocculation test, hippuric acid synthesis and the 5 mg. serial bromsulphalein test.

3. Hepatic dysfunction was demonstrated in a large percentage of cases.

4. The serial bromsulphalein test showed the greatest number of positive results.

5. The most outstanding abnormalities were found in 2 patients with complicating amyloidosis.

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THE ESOPHAGEAL VARIX

A Report of One Hundred and Fifteen Cases

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Esophageal varices have always been a dreaded complication of patients with diseases causing increased pressure in the portal vein. These varices, although often silent, insidious and symptomless in development, frequently rupture suddenly and control of the hemorrhage is usually unsuccessful. Recent advances in the early treatment of this disease give some hope of changing its relentless progress toward eventual rupture and exsanguination to possible cure and eradication of the varix. An understanding of the pathogenesis of this condition, its incidence, and the utilization of the means of early diagnosis and prompt institution of treatment are most important in making this possible.

Pathogenesis. Kegaries[6] demonstrated that a communication exists between the portal and caval system by way of the esophageal veins: they have at one end anastomoses with the splenic and coronary veins of the portal system and at the other with the azygos, intercostal and diaphragmatic veins of the systemic circulation. The absence of valves in the coronary vein makes a reversal of direction of blood flow possible, so that in portal hypertension the blood tends to dam back and enter the systemic circulation by way of the esophageal anastomoses. The damming back of blood from the portal system through the coronary vein overtaxes the capacity of the esophageal veins and in this manner varicosities begin. Other factors which contribute to their development are the poor support which the esophageal veins receive by the loose connective tissue in the esophageal submucosa and the sucking action of the diaphragm during respiration. The anatomic location of esophageal varices constantly exposes them to trauma which may lead to fatal bleeding.

The tributaries of the portal vein constitute the venous drainage from the gastrointestinal tract, the gall bladder, the pancreas and the spleen. Portal hypertension may take place as result of block of this venous return occurring within the liver as in cirrhosis, or extrahepatically along part or all of the portal system. Whipple[15] speaking of portal hypertension has said: "If there is a high retention of the bromsulphathalein in the blood 30 minutes after intravenous injection, if the hippuric acid test is positive, if there is a reversal of the albumin globulin ratio or if the cephalin flocculation test is positive, the presence of a cirrhosis with intrahepatic portal block is fairly certain. On the other hand, if these tests are negative it is safe to assume that block is extrahepatic." Extrahepatic block is most commonly due to chronic congestive splenomegaly (Banti's syndrome or to splenic vein thrombosis.

Diagnosis. Early diagnosis of the esophageal varix has always been exceedingly difficult and usually the disease is unrecognized until a patient with cirrhosis of the liver or splenomegaly has 1 or more episodes of hematemesis. Frequently it is not suspected and is found at the autopsy table or by coroners investigating causes of sudden death. In 1925 Chevalier Jackson[5] suggested esophagoscopy as an aid in diagnosis, but this method has not been widely used for fear of producing fatal hemorrhage by abrasion of the varices during instrumentation. With careful use, however, esophagoscopy is justified and there is minimal risk. Varices are usually demonstrated with ease by this

procedure and sometimes they are so large that they appear to occlude the lumen of the esophagus (Fig. 1).

In 1928 Wolf[17] first demonstrated esophageal varices by the use of Roentgen rays and these observations have been confirmed many times. The principle of Roentgen ray visualization depends upon the bulging of the dilated veins into the lumen of the esophagus causing an uneven worm-like appearance along the inner surface (Fig. 2). To demonstrate these one must coat the esophageal lumen with a thin layer of barium mixture. A thick mixture of barium is usually not satisfactory. The varices, as a rule, are smaller when the patient is in the upright position and the examination is best performed when the patient is horizontal and "bearing down." Sometimes they are demonstrated at fluoroscopy but usually "spot" films are necessary. One must remember that var-

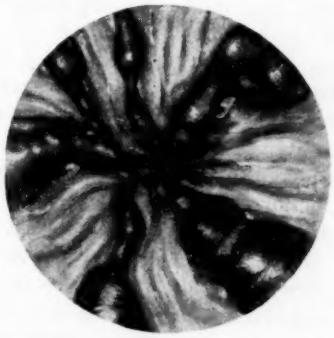

FIG. 1.—Esophageal varices as seen through an esophagoscope.

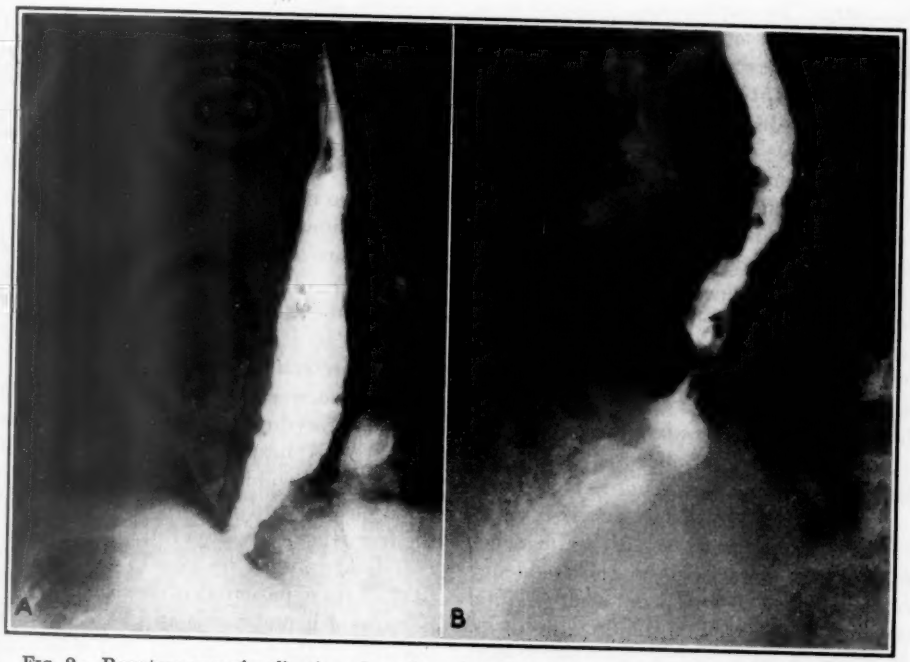

FIG. 2.—Roentgen ray visualization of esophageal varices with opaque suspension in 2 patients.

ices are compressible anatomic formations and various factors such as peristalsis, position and respiration may influence their filling.

In the differential diagnosis of the Roentgen ray picture, according to Plotz and Reich,[11] one should rule out food particles, polyps, new growths and cardiospasm. Repeat examinations after thorough evacuation of food will usually eliminate the possibility of food particles. Polyps may be ruled out because in this condition there is a rapid passage of barium and an absence of obstruction in proportion to the degree of protrusion. It is important to differentiate new growths of the esophagus and these often are ruled out by the softness of the esophageal wall and its elasticity which appears unimpaired. Esophagoscopy is sometimes necessary. In cardiospasm there is usually a smooth esophageal wall unlike that when varices are present.

Careful roentgenographic study of the esophagus is indicated in all persons who show evidence of portal hypertension. It is to be remembered that negative Roentgen ray or fluoroscopic findings do not rule out the possibility of varices and esophagoscopy may be necessary.

There is some question as to the advisability of these procedures in patients who have already had episodes of hematemesis and individual consideration must be given each case. When there is no evidence or history of recent bleeding, Roentgen ray examination and esophagoscopy are unlikely to promote new hemorrhages.

INCIDENCE. There is considerable variation in the literature as to the incidence of esophageal varices and the frequency of hemorrhage from them, whether fatal or not. In 1900, Preble,[12] in a study of 60 cases of fatal gastro-intestinal hemorrhage occurring in patients with cirrhosis of the liver, found esophageal varices in 80% and in half of these was able to demonstrate rupture of the varix. McIndoe[8] reporting 26 deaths due to cirrhosis found ruptured esophageal varices in 50% of them. Rivers and Wilbur[13] reviewed 668 patients who came to the Mayo Clinic with a primary complaint of hematemesis and found that bleeding was attributable in 5.1% of the cases to cirrhosis of the liver or splenic anemia.

In this study 115 cases of esophageal varices occurring since 1919 at the San Francisco City and County Hospital (Department of Public Health, City of San Francisco) are reported. Of these cases, 84 had proven esophageal varices by Roentgen ray, esophagoscopy, or autopsy. The remaining 31 were so diagnosed clinically because of strongly suggestive history, physical and laboratory findings and clinical course. It should be noted that difficulties in reporting statistics of this disease are especially great because patients are frequently admitted in shock and die before an adequate history can be obtained and the diagnosis is made at the autopsy table; whereas in other cases where a history, physical examination, laboratory studies and course all indicate cirrhosis of the liver or chronic congestive splenomegaly, perhaps with esophageal varices, an autopsy is not obtained or the varices are not demonstrated at postmortem examination. The 31 clinically diagnosed cases of esophageal varices were of this group. The inability to demonstrate esophageal varices at autopsy is well known. It is generally conceded that many more could be demonstrated if contrast media were injected to detect them but this procedure is not the general practice at this hospital. Consequently at best one is only able to acquire the approximate incidence of esophageal varices and the frequency of related fatal hemorrhage.

It is of interest to note (Table 1) that 61% of these patients entered the hospital because of hematemesis and 84% of these (or 51% of the total group) died of hemorrhage. Only 18% of those studied gave a history of previous episodes of hematemesis, which gives a rather ominous outlook for the patient with esophageal varices when he first vomits blood. There was a history of excessive alcoholism in 61% and in 80% cirrhosis of the liver was

either diagnosed or found at autopsy (Table 2). Other causes of esophageal varices resulting from portal hypertension included chronic congestive splenomegaly, gumma of the liver, portal vein thrombosis and tumors of the liver. The age of the patient varied from 26 to 80 years, the average of the whole group being 50.

tion or spider angiomata in the cases studied (Table 3). The incidence of peripheral neuritis was surprisingly low in a group composed so largely of alcoholics.

At best, then, one can say that esophageal varices are a frequent complication of portal hypertension and it is with just cause that both patient and doctor await

TABLE 1.—MORTALITY OF PATIENTS WHO ENTERED COMPLAINING OF HEMATEMESIS AND THE INCIDENCE OF PREVIOUS HEMORRHAGES IN THE 115 CASES OF ESOPHAGEAL VARICES STUDIED

	Group A (84 proven cases)	Group B (31 clinically diagnosed cases)	Total (115 cases both groups)
Presenting complaint: hematemesis	♂ 32 ♀ 24 56 (66%)	♂ 10 ♀ 4 14 (45%)	Both sexes 70 (61%)
Previous history of hematemesis	♂ 11 ♀ 4 15 (18%)	♂ 9 ♀ 1 10 (32%)	Both sexes 25 (22%)
Died of hemorrhage	♂ 31 ♀ 18 49 (59%)	♂ 7 ♀ 3 10 (32%)	Both sexes 59 (51%)

Mortality of total group of patients who entered with presenting complaints of hematemesis: 59 of 70 (84%).

TABLE 2.—AVERAGE AGE, INCIDENCE OF CIRRHOSIS AND HISTORY OF ALCOHOLISM IN 115 CASES OF ESOPHAGEAL VARICES STUDIED

	Group A (84 proven cases)	Group B (31 cases clinically diagnosed)	Total (115 cases)
Average age	♂ 50 ♀ 47	♂ 54 ♀ 49	50 years
Incidence of cirrhosis	♂ 40 ♀ 23 63 (75%)	♂ 20 ♀ 9 29 (94%)	92 (80%)
History of alcoholism	♂ 26 ♀ 20 46 (55%)	♂ 16 ♀ 8 24 (80%)	70 (61%)

The vast majority of the patients had an unexpected sudden onset of hemorrhage usually unrelated to stress, cough or eating. In 4 the bleeding occurred while the patients were asleep and they woke up with feelings of nausea and vomited blood. Five of the patients noted the onset of their hematemesis following a blow to their abdomen or thorax and several more associated their onset with coughing. There seemed to be no relation between the occurrences of varices and the presence of an enlarged liver or spleen, jaundice, ascites, edema, collateral nervous circula-

TABLE 3.—OCCURRENCE OF MISCELLANEOUS PHYSICAL FINDINGS OF THE 115 PATIENTS WITH ESOPHAGEAL VARICES

	No. cases
Enlarged liver	81
Jaundice	38
Ascites	37
Edema	18
Palpable spleen	20
Collateral venous circulation	24
Peripheral neuritis	8
Spider angiomata	23

with alarm and fear their development and eventual rupture. Such a poor prognosis should be sufficient reason for a relentless search for a method of eradica-

tion and treatment of this situation, generally thought hopeless.

TREATMENT. Until quite recently the treatment of esophageal varices has been very discouraging. Medical treatment has been more directed at the underlying condition. In patients with cirrhosis of the liver diets high in protein, carbohydrate and vitamins and the use of liver concentrates have been of some value in improving liver function, and this probably reduces the likelihood of varices developing. Mercurial diuretics have been only palliative. Drenckhahn[3] suggested that blood with a lower viscosity might pass more easily through the liver since it presented less obstruction and in this way the portal pressure would be reduced. He found that some patients who had frequently bleeding esophageal varices bled when the total red blood count was close to normal. The blood at this time is considerably more viscid than when there are about half the normal number of red blood cells present. He reported the successful treatment of 1 patient whose blood he kept at a low viscosity by repeated venesections. This method, however, has not received general use.

Treatment of the reported cases of this paper was only palliative and was entirely unsatisfactory. All of the patients were treated for shock, sedated, kept at bed rest, and dietary precautions were observed. About half were given plasma or whole blood transfusions during the bleeding phase. Some authorities maintain that the increase in blood volume which occurs during transfusion is unwise during a hemorrhage in that the tear in the vascular wall is less likely to close at this time. However, we noticed no significant difference in the group who received transfusions.

More favorable results have been obtained by surgical intervention. Removal of the spleen is perhaps the most common surgical procedure although some observers report recurrences of hemorrhages from esophageal varices following splenectomy.[1] Omentopexy has been done to establish collateral circulation around the liver through the veins of the peritoneum and the abdominal wall, especially through the superior epigastric veins. Interrupting the flow of blood to the esophageal veins by ligation of the coronary vein with or without splenectomy as described by Rowntree, Walters and McIndoe[14] produced favorable results in some cases. Moersch in 1941[7] reported the injection of sclerosing substances into the varices under direct vision with the use of the esophagoscope but thought this method should follow splenectomy. Patterson and Rouse[9] reported in 1946 injecting the esophageal varices of 8 patients with 5% sodium morrhuate. Two later died from bleeding varices. It is interesting that they treated successfully by injection the bleeding varices of 1 patient while he was in shock from loss of blood and were able to stop the bleeding. The success of this treatment, although heroic in nature, should be borne in mind when one is confronted with similar patients who are moribund from bleeding esophageal varices. Neither vein ligation nor the injection treatment *per se* of the esophageal varices is physiologically sound, for unless other methods are used to reduce the portal hypertension the varices most likely will recur.

A more physiologic approach to the treatment of the esophageal varix is that of lowering the pressure in the portal vein which in turn will decrease the pressure in the esophageal veins. This can be done by anastomosing the portal and caval circulations. In 1877 Eck[4] first suggested this as a method of sidetracking the venous return in obstruction of the portal vein. The Eck fistula procedure has been tried occasionally since then, but the high operative mortality discouraged its general use. The recent developments in vascular surgery has renewed the interest in this procedure. In 1946 Blakemore and Whipple[1,16] reported portocaval anastomotic procedures on 14 patients, 4 with extrahepatic portal obstruction and 10 with intrahepatic obstruction due to portal

cirrhosis. There were 2 postoperative deaths, both among the cirrhosis group, but the remainder were considerably improved, and had little or no gastro-intestinal bleeding postoperatively. Several had marked reduction in ascites.

Blalock[2] recently has supported the portocaval shunt operation in the treatment of ascites and gastro-intestinal bleeding as being the most sound of any of the methods of treatment so far advocated and has done this procedure on selected cases. The technical skill required for the operation is considerable and its success depends upon the portocaval junction not breaking down and remaining patent enough for an appreciable amount of blood to by-pass the liver.

It is hoped that further experience accompanied by good results will popularize this first physiologic attack on a serious problem so far unsolved.

Summary. Of 115 patients with esophageal varices reported here, 70% entered the hospital with the presenting complaint of hematemesis and 84% of these died of hemorrhage shortly after admission. A previous history of hematemesis was elicited in 22%. Other factors including the incidence of cirrhosis, alcoholism, age and physical findings of these patients are presented. The pathogenesis and methods of diagnosis of esophageal varices are described and the various forms of treatment including the recent work on portocaval anastomoses are discussed.

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THE ACTION OF PENICILLIN ON TREPONEMA PALLIDUM*

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ALTHOUGH it is just a little more than 4 years since Mahoney, Arnold and Harris[34] demonstrated that penicillin was effective treatment for early syphilis in rabbits and man, a voluminous literature has appeared on the value and limitations as well as possible mechanism of this action. Since the first trials of penicillin in syphilotherapy, many new developments have conditioned the degree of this antispirochetal effect. Commercial penicillin has been shown to be a mixture of several types, named in this country F, G, X and K, each, as well as the commercial product, with its own pharmacologic action against spirochetes *in vitro* and *in vivo;* impurities in the original mixtures have an activity against spirochetes; the individual penicillin types in the crystalline state act differently from mixtures containing varying amounts of the various types, and lately penicillin has been synthesized, a development which as yet has yielded no opportunity for those interested in syphilotherapy to take advantage of. It has also been demonstrated that penicillin has similar potency against other spirochetal infections such as relapsing fever,[16d,24a,47,51] yaws,[14,32,53,62] Weil's disease,[1,3,24b] Vincent's infection,[37,48,50] and rat-bite fever.[24c,27,33,46] Employment of penicillin in these diseases may yield rapid methods for determining the value of various penicillins against syphilis. In addition to the group specific action of penicillin against various spirochetes, it apparently has a varied effectiveness against virulent *Treponema pallidum* as contrasted with avirulent cultured organisms of so-called Reiter, Nichols and Kazan strains. In spite of careful evaluation of the many factors defining the mode of action of penicillin on spirochetes, especially *T. pallidum*, the exact mechanism and site of action are little understood. However, because of the industry of a group of laboratory investigators (Eagle, Mahoney, Chesney, Magnuson, Fleming, Rosahn, Levaditi, Carpenter, Rake and their associates), sufficient data on various aspects of the problem of the effect of penicillin on *T. pallidum* are available for review. We intend to discuss this material under the following heads: The activity of penicillin against *T. pallidum in vitro*, including commercial penicillins F, G, X and K;

* *Treponema pallidum* and *Spirochæta pallida* are used interchangeably as the designation for the organism alleged to cause syphilis.

the effect of penicillin in experimental rabbit syphilis, including the effectiveness of commercial penicillin, penicillins F, G, X and K and impurities of commercial penicillin; resistance to penicillin; and the mode of action of penicillin on *T. pallidum*.

At present, information on the various topics listed above is limited in many respects and, as is usually the case, results from *in vivo* animal studies and *in vitro* experiments cannot be directly applied to the problem of human infection with syphilis. Although there is in general a rough parallelism between the *in vitro* activity of penicillin against various organisms and its clinical use in infections caused by them, various factors in animals and man, variations in absorption, excretion, the presence of inhibitors and the complex nature of the difficult to cure disease, syphilis, etc., may cause this relationship to break down in specific instances. Another *desideratum* for the future study of the effects of penicillins on *T. pallidum* is a standardized preparation with dosage in milligrams and not in terms of potency against a particular organism unrelated to that alleged to cause syphilis. In this connection, Moore[39b] has aptly stated that "To express dosage in syphilis, caused by the *Treponeme pallidum*, in terms of staphylococcus units determined *in vitro* is, to say the least, a *non sequitur*.

"What appears to be needed, therefore, now that the honeymoon is over, is a systematic and painstaking study of the following:

"1. Exact definition of the basic chemical structure of penicillin, and if possible, its synthesis.

"2. Separation from commercial penicillin of its various species, and their preparation in pure crystalline form.

"3. The expression of dosage in gravimetric forms, *i. e.*, milligrams in place of units.

"4. The chemical synthesis of new modified penicillin.

"5. The rate of absorption and excretion of each penicillin as prepared in crystalline form.

"6. The effectiveness, both *in vitro* and *in vivo*, of each crystalline penicillin against various bacteria.

"7. Toxicologic, pharmacologic, and therapeutic study of each crystalline penicillin."

THE ACTION OF PENICILLIN ON T. PALLIDUM IN VITRO. In 1944, Eagle and Musselman[17a,b] made an extensive study of the spirocheticidal action of commercial penicillin *in vitro* and its temperature coefficient. In view of the fact that pathogenic spirochetes have as yet not been cultured, recourse was had to non-pathogenic strains, especially the so-called Reiter strain. Penicillin was found to be actively spirocheticidal *in vitro* (direct spirocheticidal effect) against the Reiter, Kazan, Nichols and Noguchi strains of *T. pallidum* and a strain of mouth spirochetes. The threshold concentration was 0.01 unit per cc. (1:160,000,000 penicillin). The rate and degree of action increase with the concentration of penicillin up to a level of 0.1 to 0.25 unit per cc., which rendered more than 99% of the organisms non-viable within 12 hours. Higher concentrations did not appreciably accelerate the effect. Within limits (4×10^4–10^7 organisms per cc.) the initial rate at which the spirochetes were killed was not affected by this number but the amount of penicillin required to sterilize suspensions of varying density varied to a large extent with the initial number of organisms. It was found that the organisms remained actively motile for a period of 8 to 24 hours after they had been rendered non-viable by the action of penicillin. Higher concentrations (500 units of penicillin per cc. or approximately 10,000 times an effectively spirocheticidal concentration) did not accelerate that delayed immobilization. Thus, although penicillin rapidly renders organisms non-viable, the metabolic system affected is not immediately essential to the life of the cell, and the motility and presumably other vital functions remain unaffected for a significant number of hours. The rate at which organisms were killed by penicillin increased with tem-

perature in the range of 8° to 40° C. Of an original inoculum of 10^6 spirochetes per cc. exposed to penicillin, the percentage of organisms surviving after 24 hours was 100 at 80° C.; 10 at 22° to 23° C.; 1 at 32° to 33° C.; and only 0.02 at 30° to 40° C. These results were independent of the concentration of penicillin in the range of 0.25 to 250 units per cc. They suggest that the combined use of penicillin with fever in the treatment of syphilis may be more effective than either one alone. Lee and Foley[28] showed *in vitro* that with other organisms (not spirochetes) within the range of 37° to 42° C., the action of penicillin is enhanced by increased temperature. They found that the action is furthermore far greater on organisms undergoing rapid rate of growth. At high temperatures the drug kills organisms that are not undergoing growth, an effect which would otherwise require excessively high concentrations of the drug. The same synergistic action of fever and penicillin has been noted by Hoyt, Pratt and Levine.[25] Dunham, Hamre, McKee and Rake,[12] employing the technique of Eagle,[15d] using Nichols strain of *Spirochæta pallida*, obtained from rabbit testes in the stage of acute orchitis, found penicillin to be spirocheticidal when relatively high concentrations (800 to 1600 Oxford units per 0.8 ml.) were used. Bessemans and Derom,[5c] confirming an earlier experience[5a] and using fragments made of about 1 c.mm. of testicular syphiloma of the rabbit rich in spirochetes and physiologic emulsions homogenized by gentle centrifugation so that there were about 2 to 3 motile treponemas per field, found that after 3 hours of action at 37° C. penicillin was treponemicidal *in vitro* in a concentration of 10,000 units per cc. A concentration of 1000 units produced partial action as judged by the retarded appearance of lesions in rabbits' testes. The state of the pathogenic material did not influence the results; the penicillin seemed well diffused in the tissues. Moore[39b] cites unpublished work of Eagle to the effect that exposure of pathogenic treponemas to the commercial penicillin, in a concentration of 64 units per cc. at 37° C. for periods of from 8 to 24 hours, did not impair their infectiousness. "This paradoxical finding, inconsistent either with the direct spirocheticidal action of penicillin on cultured spirochetes, or its effect on pathogenic *Treponema pallidum in vivo*, has not yet been explained."

The effect of penicillin *in vitro* on spirochetal and other microbic morphology, observed by darkfield on the electron microscope, has been reported by various investigators. Details and the possible significance of their findings will be discussed below.

Commercial penicillin contains at least 4 molecular species (F, G, X and K in this country, and penicillin I, II, III and K respectively in Great Britain). They differ from each other in the nature of the side group attached to a common nuclear structure. These penicillins are known to vary in their bactericidal activity *in vitro* (Ory *et al.*,[44] Veldee *et al.*,[58] Welch *et al.*,[61] Libby *et al.*[31]) Compared with penicillin G, rated as 100, the relative activities per mg. of penicillin F, G, K and X against *Staphylococcus aureus* are reported to be 90, 100, 140 and 55 respectively (1550, 1667, 2300 and 900 units/mg.). Eagle and Musselman[17c] found that crystalline specimens of F, G, K and X have a relative gravimetric activity *in vitro* of 82, 100, 120 and 140 against the C-203 strain hemolytic streptococcus, and 53,100, 75 and 50 respectively, against a cultured strain of *Spir. pallida* (Reiter).

In addition to the 4 forms of penicillin in the commercial product, there are different amounts of other substances of unknown nature. As was the case with the arsenicals, purification of penicillin has resulted in a somewhat lessened antispirochetal activity. This is suggested by the larger and larger dosages of penicillin salts needed for the control of clinical syphilis. The possibility that these impurities may have antispirochetal properties is suggested by the observations of Dunham and Rake[13] who found that while partially

purified penicillin immobilizes *T. pallidum in vitro* (Dunham, Hamre, McKee and Rake), crystalline penicillin G has very little or no effect on the motility of *T. pallidum* when the latter is exposed to 8800 u./ml. for 2 hours *in vitro*. Under the same conditions, a solution containing 2200 u./ml. of the least pure penicillin preparation employed in their studies, immobilizes all of the spirochetes. A substance in partially purified penicillin that immobilizes spirochetes can be concentrated by adsorption on alumina and recovered by elution. The concentration of the substance in partially purified penicillin that is active against spirochetes *in vitro* is only slightly, if at all, reduced by incubation at 37° C. in the presence of a weak solution of penicillinase over a period of 11 days, conditions under which a large proportion of the penicillin present is inactivated.

Dunham and her associates concluded that the immobilization of spirochetes *in vitro* by partially purified penicillin is due to one or more of the impurities present. No marked degree of immobilization of spirochetes is produced by solutions containing 3.5 mg./ml. of penillic acid or 30 mg./ml. of a product of the hydrolysis of pure penicillin G. They found in addition that spirochetes exposed to 1100 u./ml. of certain partially purified preparations of penicillin are non-infectious for rabbits when injected intratesticularly, although many of the organisms were still motile at the time of injection. Spirochetes similarly exposed to 8800 u./ml. of crystalline penicillin G (8 times as much) produces orchitis in rabbits. The comparative findings of these authors in experimental syphilis in rabbits will be discussed later.

THE ACTION OF PENICILLIN ON T. PALLIDUM IN VIVO.* As noted previously, the results of *in vitro* studies of the antispirochetal action of penicillin can be referred only in a general way to the effects of the class of preparations *in vivo* (animals and man). Still, the efforts of the group of laboratory workers noted above have yielded information which may lead to a better understanding of the therapeutic problem in man.

Shortly after Mahoney, Arnold and Harris[34] showed that penicillin was active against syphilis in animal and man, other studies began to appear. Several of these (Selbie and Simon;[49] Ercoli and Lafferty;[18] Raiziss[45]) dealing with a small number of animals (less than 10) indicated that penicillin was of little or no value in experimental rabbit syphilis. For example, Ercoli and Lafferty found that a single intravenous dose of penicillin sodium—33,000 to 47,000 u./kg. or 16,000 u./kg. if the drug were given over a period of 72 hours in doses of approximately 4000 u./kg.—was required to produce disappearance of *T. pallidum* in experimental rabbit syphilis. Lymph node transfer in 4 rabbits, however, indicated that the highest doses used (132,000 to 282,000 u./kg.) did not sterilize the rabbits. Selbie and Simon likewise found that 45,000 u./kg. when given to rabbits in 3 equally divided doses at intervals of 3 hours, caused the disappearance of organisms within 24 hours, as determined by the direct examination of testicular lesions. Organisms, however, reappeared in the majority of the syphilomata in from 30 to 45 days after treatment. In view of the clinical experience and other later studies, these early investigators did not take into account the factor of time-dosage relationships necessary for cure with syphilis. (McDermott[36] and his colleagues found, based on available *in vitro* and *in vivo* data, that "it would seem that a serum penicillin concentration of 0.078 unit per cc. is probably well above the 'effective level' for the spirochete.)" In addition, their results are clear demonstration of the unreliability

* A number of studies, not yet in print, have been publically presented in a symposium, Recent Advances in the Investigation of Venereal Diseases, in Washington, D. C., on April 17, 1947, under the Syphilis Study Section of the National Institute of Health. Since these data are not officially available, they are not included in this review.

of disappearance of spirochetes from lesions as evidence of cure (Beerman[4]). Even small doses of penicillin will readily cause surface sterilization in clinical syphilotherapy (Binkley and Kile;[6] Moore and co-workers[40]). Dunham, Hamre, McKee and Rake in 1944[12] furthermore found that inadequate dosage in rabbit syphilis resulted in the production of a more resistant strain of *T. pallidum*. Bessemans and Derom,[5b] on the other hand, reported that rabbits and mice were completely sterilized when given relatively large doses of penicillin, namely, a total dose of 320,000 Oxford units (5000 units injected intramuscularly at 3 hour intervals in 8 days) for the rabbits and 12,800 units for 8 days and 18,000 units in 1.5 days for the mice.

In a later communication, Bessemans and Derom[5c] reported new results of penicillin therapy of rabbits and mice. They observed that complete sterilization as measured by tissue transfer occurred in mice with a total dose of 800,000 u./kg. in 8 days at the onset of the infection, and 1,200,000 u./kg. in 11 days in the old cases. In the rabbit the good results were obtained when the treatment was given in doses totalling 100,000 u./kg. in 8 days or of 250,000 u./kg. in 19 days, either as injection in physiologic saline every 3 hours or every 12 hours as an emulsion in oil (according to the procedure of Romansky and Rittman). Earlier (1944) Levaditi and Vaisman[30] stated from their experiences, in preliminary studies, that penicillin injected either locally or intravenously in variable numbers of multiple doses but with total dosage of 7500, 10,000 and 80,000 Oxford units, caused rapid disappearance of spirochetes and healing of lesions in rabbit syphilis (Gand strain) in a short time. Curative action clinically speaking was certain. The question of complete sterilization by penicillin had not been determined. In mice with asymptomatic infections, 750,000 Oxford u./kg. body weight were needed to cause complete sterilization. In 1945 they demonstrated that penicillin given intravenously in rabbits in total doses ranging between 500 and 40,000 u./kg. at 1 to 3 injections daily, caused a rapid curative effect from the standpoint of disappearance of spirochetes and lesion healing. However, when given intramuscularly in total mass of 40,000 Oxford u./kg. in 20 doses in 4 to 5 days, they noted that penicillin caused not only rapid disappearance of spirochetes and clearing of the lesions, but complete sterilization of the animal's syphilis. Total dosage and rhythm of the injections seem to play an important rôle in the results obtained. In addition, these investigators noted that penicillin given intramuscularly in dosage of 6000 to 40,000 Oxford u./kg. caused rapid disappearance of spirochetes from rabbit syphilomas as determined by darkfield, but silver stains for tissue spirochetes were positive and spirochetes were present in great numbers in the lesions. Certain of them showed involution forms, the loss of pathogenic growth preceding total resorption of the organisms resembling the effects of bismuth and arsenicals.

Fleming and Wolf[21] employed an aqueous solution of a relatively crude sodium penicillin (2930 per mg.); 25% penicillin X in early and late rabbit syphilis (duration 6 weeks and 6 months after infection respectively) in a schedule of intramuscular injections every 3 hours day and night for 4 days (32 injections) in 5 total dosages ranging from 500 to 80,000 u./kg. Using Eagle's criteria of cure and 10 rabbits for each of the schedules, they found that the CD_{50} (dose curing 50%) in early syphilis was 1100 u./kg.; the CD_{95} dose (curing about 95%) was 2000 units. In late syphilis the corresponding figures were approximately 500 and 2000 u./kg. These data indicate that late rabbit syphilis is as curable as the early infection and perhaps with even a somewhat smaller dose. Moore[39b] cites experimental data of Arnold (unpublished) which suggest that in the rabbit, at any rate, cure of syphilis with penicillin can be accomplished in a short time (12 hours) by enormously increasing the total dose, to

perhaps 400,000 u./kg., the equivalent of 28,000,000 units in a 70 kg. man. Experiments of this sort have been carried out by Schwemlein and his associates who have treated 162 patients with primary or secondary syphilis by continuous intravenous drip of from 10,000,000 to 25,000,000 units of penicillin over a 24 hour period. Their clinical results have not as yet been published.

Not only is penicillin curative in 3 days in rabbit syphilis but Arnold, Mahoney and Cutler[2a] have demonstrated that rabbits with acute syphilis given adequate penicillin therapy were reinfected 10 days after treatment was completed. Untreated rabbits with acute syphilis reinoculated with an homologous strain of spirochetes at the same time period as the treated animals did not develop clinical evidence of reinfection.*

From this listing of variable results of penicillin therapy in rabbits and mice, mostly favorable, one turns to the illuminating and brilliant studies of Eagle and his co-workers. Eagle has recognized the variable factors which have to be studied in order to bring order out of the chaos of penicillin therapy. Because these variable factors can be controlled in animals (especially the rabbit) with more certainty than in man, the experimental animal is a better subject for study than man. Moore[39b] has clearly summarized the manifold variables involved as follows: "As to commercial penicillin used alone, and injected intramuscularly in aqueous solution, these variables are the number of injections, their frequency and the total amount of the penicillin administered. Equally important is a study of absorption delaying methods (*e. g.*, calcium penicillin in peanut oil-beeswax) with the adjuvant use of arsenic, bismuth or fever. Of still greater fundamental importance is a study of penicillin species and impurities present in commercial penicillin, to determine which of the several components is most, or perhaps solely, active. All of these studies are under way, the latter temporarily hampered by lack of precise chemical information and production difficulties." In statements which follow we shall outline briefly the published results of Eagle and his co-workers and interpolate data from the observations of others.

Effect of Method of Administration on the Efficiency of Sodium Penicillin in Experimental Syphilis. This aspect of the problem has been thoroughly studied by Eagle, Magnuson and Fleischman.[16c] They used rabbits recently successfully injected intratesticularly (5 to 7 weeks) with the Nichols strain of *T. pallidum* and injected 9 different lots of penicillin varying in purity from 130 to 1025 Oxford u./mg. in accordance with 13 different schedules on which the interval between injections varied from 15 minutes to 4 days, the total number of injections from 4 to 50, and the duration of treatment from 3 hours to 16 days. In each of the schedules, 3 to 9 rabbits were treated at each of 3 to 7 dosage levels, which usually varied in 2-fold steps. Since no clear-cut differences were noted in the lots of drugs studied, the various brands of penicillin were not compared. Cure was determined by immediate observations on the disappearance of spirochetes and by lymph node transfers at proper intervals. The results of these experiments indicated that the curative dose of sodium penicillin in rabbits was affected to a striking degree by the number of injections. The greater that number, the less was the *total* quantity of penicillin required for cure. On the other hand, they noted that, provided only that the interval between injections was sufficiently great to avoid cumulative effects on the blood penicillin level (in which case therapeutic efficiency was paradoxically diminished), it made no difference whether penicillin was administered every 4 hours, twice daily or daily; an equal number of injections at a given dosage level produced equivalent effects. The relationship of blood levels and the therapeutic results of penicillin are under study; although the therapeutic action of penicillin clearly involves both the tissue concentrations and the time over which

* These same authors[2a] observed similar results in early latent syphilis (8 months' durations in rabbits).

they act, the latter time factor is by far the more important. Low concentrations acting over a long period of time (*i. e.*, many small injections) were more effective than high concentrations acting over a short period (namely, a few large injections to the same total dose). Within the time limits of their experiments, the interval between injections was immaterial, provided they were not given too often: for an equal number of injections, treatments once daily were as effective as injections every 4 hours, with a suggestion of an optimum interval of 8 to 12 hours.

On the basis of the present experimental data, Eagle and his colleagues believe that it may be anticipated that the results in the treatment of human early syphilis with sodium penicillin could be significantly improved by (*a*) prolonging the duration of treatment, (*b*) increasing the frequency and number of injections, and (*c*) increasing the total dosage of penicillin. The use of a suspension of calcium penicillin in oil and beeswax, or the administration of penicillin in a continuous intravenous, intramuscular or subcutaneous drip, or any other procedure which delays the absorption and excretion of penicillin would have the same effect as increasing the frequency and number of injections.

They feel that there is reason to believe that the treatment of syphilis with sodium penicillin need not be carried out in hospitalized patients, but that it may be given on an ambulatory basis once or twice daily without necessarily sacrificing therapeutic efficacy, provided only that the patient receives the requisite total number of injections. The use of calcium penicillin in oil and beeswax, or of similar devices to delay the absorption and excretion of penicillin, may permit treatment to be given as infrequently as twice weekly, and perhaps even irregularly within that time interval.

The total curative dose (CD_{90}) of penicillin on the best schedule here tested with 500 u./kg. or approximately 0.3 mg./kg. of penicillin G. Milligram for milligram, penicillin is therefore 10 to 20 times as effective as mapharsen in the treatment of rabbit syphilis. In the human infection, however, penicillin is apparently only 2 to 4 times as active as mapharsen, milligram for milligram.

With either penicillin or mapharsen, rabbit syphilis is easier to cure than the human disease, requiring less than one-fourth to one-eighth as much mapharsen, and less than one-twentieth to one-fortieth as much penicillin.

In experiments designed to test the effect on therapeutic efficacy in experimental syphilis of delaying absorption of penicillin as postulated above by Eagle, Magnuson and Fleischman,[16c] this same group[16b] found that their deductions regarding the influence of delaying absorption and thus prolonging blood levels on curative action was correct. Using rabbits treated 5 to 7 weeks after testicular inoculation with 9 different lots of penicillin (4 were commercially prepared suspensions of the calcium salt in peanut-oil and beeswax) it was found that the curative dose (CB_{50}) of the oil-beeswax mixture, given daily for 4 days, fell from 39,000 to 8000 to 3500 u./kg. as the percentage of beeswax increased from 0 to 3 to 6% (by volume). The corresponding CD_{90} values were 80,000, 16,000 and 8000 u./kg. When calcium penicillin in oil and beeswax (4.8 to 5.3% by weight) was administered (*a*) as a single injection, (*b*) twice at 8 hour intervals, (*c*) daily for 4 days, (*d*) twice daily for 8 days, and (*e*) twice weekly for 8 weeks, the CD_{50} dose was, respectively, (*a*) 50,000, (*b*) 54,000, (*c*) 3500, (*d*) 800 and (*e*) 1500 u./kg. The corresponding CD_{00} doses were as follows: (*a*) 100,000, (*b*) 90,000, (*c*) 8000, (*d*)1800 and (*e*) 2000 u./kg. In contrast the CD_{50} of penicillin in aqueous solution given (*a*) as a single massive injection, (*c*) daily for 4 days, and (*d*) twice daily for 8 days was (*a*) greater than 600,000, (*c*) 50,000, and (*d*) 1770 u./kg. respectively (that is, 2 to 12 times the curative dose of the oil-beeswax preparation similarly administered). The curative dose of penicillin, aqueous solution or oily suspension, varied

with the number of injections into which the treatment was divided. They found that if one compares the efficacy of the 2 preparations on the basis of number of injections rather than the dosage: (*a*) to cure syphilis in 1 day with a total of 50,000 units required 1 injection of the oil-beeswax preparation, and 12 injections of the aqueous solution; (*b*) to cure syphilis in 4 days with a total of 3500 u./kg. required 4 injections of the oil-beeswax preparation and 16 of the aqueous solution; (*c*) to cure syphilis in 8 days with 800 u./kg. required 16 injections of the oily preparation and 32 of the aqueous solution. Thus the total dose of aqueous solution had to be divided into 2 to 14 times as many injections as the oil-beeswax suspension in order to be equally effective. This superiority of the oil-beeswax suspension was especially striking in schedules involving relatively large injections. The smaller the individual dose of penicillin, the less pronounced was the margin of superiority. Kolmer and Rule[26a] also studied the therapeutic activity of penicillin in single and multiple doses in isotonic solution of sodium chloride and peanut oil-beeswax by intramuscular injection. They found that in the treatment of acute syphilitic orchitis of rabbits the minimal single curative dose of commercial and purified amorphous sodium salts of penicillin in isotonic solution of sodium chloride by intramuscular injection was more than 160,000 u./kg. of weight. For penicillin suspended in peanut oil-beeswax, the corresponding dose was 10,000 units. When given once a day for 8 days in succession, the minimum curative dose of the salt solution of penicillin was approximately 5000 u./kg. per dose, totalling 40,000 units. When given intramuscularly twice daily for 8 days in succession, it was approximately 1000 u./kg. per dose, totalling 16,000 units. The corresponding doses for penicillin suspended in sterile peanut oil and beeswax by intramuscular injection approximately 1000 u./kg. per dose, totalling 8000 units and less than 1000 u./kg. per dose or less than a total of 16,000 units. In confirmation of the results of others, they noted that penicillin suspended in peanut oil-beeswax by intramuscular injection, therefore, was found to be therapeutically more effective in the treatment of acute syphilitic orchitis of rabbits than that administered dissolved in isotonic solution of sodium chloride by intramuscular injection. The commercial penicillin employed in these experiments contained approximately 88% penicillin G and the purified approximately 92% G. Both commercial and purified lots gave essentially similar results. The above results are, in addition, a large scale elaboration of the concept described in a preliminary way with few rabbits by Raiziss in 1944[45] "that penicillin in oil suspension is therapeutically somewhat more active in experimental rabbit syphilis than penicillin in aqueous solution. The superiority of the oil suspension, however, lies chiefly in the fact that the administration of the drug can be reduced to one treatment a day."

The Effect of the Various Penicillins and Impurities in Experimental Rabbit Syphilis. Not only does dosage, method of administration, etc., play a rôle in the effect of the drug on *Spir. pallida*, but, as was indicated in the *in vitro* studies, the type of penicillin and impurities also are factors conditioning the *in vivo* action on this organism. Continuing their study of the relative activity of partially purified penicillin and of crystalline penicillin G on *T. pallidum*, Dunham and Rake[13] showed in experimental rabbit syphilis that partially purified penicillin, 330 u./kg. —protected (*not cured*) a large proportion of rabbits from local and generalized infection when 66,000 u./kg. of body weight were administered intramuscularly in the leg 5 hours after a suspension of spirochetes had been rubbed into an incision on the back; the same dose of crystalline penicillin G in another experiment failed to protect. That penicillin G, however, has spirocheticidal action *in vivo* is evidenced by the fact that 166,000 u./kg. of this preparation prevented the develop-

ment of local syphilitic lesions on a large proportion of the rabbits. On the other hand, an alumina adsorbate of partially purified penicillin showed a greater activity *in vivo* than did crystalline penicillin G. Thus, again, it is suggested that some impurities in the crude penicillin product have antitreponemal power. As stated above, Eagle and Musselman[17c] found a varying therapeutic activity against a cultured strain of *Spir. pallida* (Reiter) in crystalline samples of penicillin F, G, K and X. Subsequently they studied this problem in experimental rabbit syphilis. Their observations indicated differences in the relative activity of the several penicillins *in vivo* far exceeding those observed *in vitro*. The curative dose (CD_{50}) of commercial penicillins, which probably consisted largely of penicillin G, had been found by Eagle, Magnuson and Fleischman (cited by Eagle and Musselman[17c]) to be 1650 u./kg. when given every 4 hours for 20 injections. However, with penicillin K similarly administered, preliminary data indicated that even 16,000 u./kg. were not curative. In an attempt to assess the cause of this discrepancy Eagle and Musselman[17c] also noted that in the treatment of experimental syphilis, penicillin K has been far less efficient than other penicillin compounds. This low therapeutic activity of penicillin K has been found by them to be due to the fact that penicillin K disappears from the blood more rapidly than do the other penicillins. One hour after the injection into rabbits or man of penicillin G, F, X and K, blood levels of K were one-fourth to one-eleventh of those observed with the other penicillins, and K persisted at demonstrable levels for relatively shorter periods. The recovery of penicillin K in the urine averaged 30 to 35%, compared with an average recovery from G, F and X of 74% in rabbits and 91% in man.

These data suggest that penicillin K is inactivated in the body to a greater extent and more rapidly than penicillins G, F and X and indicate that the amount of K in commercial penicillin should be kept minimal.*

It is of interest to note that clinically, penicillin G is the preparation which seems to have the greatest effect on *T. pallidum* as demonstrated by accelerated disappearance of surface organisms from darkfield positive lesions. For example, Olansky and Putnam[43] found that penicillin G appeared equally effective as compared with several commercial penicillin preparations and penicillin X appears less effective than penicillin G in causing the disappearance of spirochetes from darkfield positive lesions. In a more extensive study, Tucker and Robinson[55] noted that penicillin G, given to 35 patients with early syphilis in amounts ranging from 0.038 to 87 mg./kg., yielded an inverse relationship between dosage of this penicillin type and the time required for disappearance of treponemes. While, as has frequently been demonstrated, disappearance of surface organisms from syphilitic lesions is a false criterion of curative action, this property of a preparation in competent hands is a useful rapid indicator of possible differences in various preparations.

Relation of the Size of the Inoculum and the Age of the Infection to the Curative Dose of Penicillin in Experimental Syphilis. Eagle, Magnuson and Fleischman[16e] have reported an extensive investigation in this problem which is important from the standpoint of chemical prophylaxis of syphilis. As previously stated, Eagle and Musselman[17b] showed that when penicillin is added to a suspension of the Reiter strain of *Spir. pallida* the number of viable organisms falls off at a rate which is largely independent of the number of organisms, and which varies with the concentration of penicillin in the range of 0.01 to 1 u./cc. If the initial number of organisms is increased, a larger proportion must be killed in order to prevent growth in subculture. Thus penicillin must be allowed to act for a longer period of time, or, the time factor remaining constant, the concentration of penicillin must be increased within the range which

* See also, Turner, Cumberland, and Li, Am. J. Syph., Gonor. and Ven. Dis., 31, 476, 1947.

affects the rate of its spirocheticidal action. These *in vitro* relationships were believed to apply to *in vivo* situations. Eagle and his co-workers showed in the present study that precisely these relationships have been found to obtain in experimental syphilis. If rabbits are inoculated with varying numbers of organisms, there is a corresponding variation in the amount of penicillin necessary to abort the infection, when administered 4 days after inoculation. Conversely if the size of the inoculum is fixed and if the animals are treated at varying intervals after their inoculation, there is a progressive increase in the amount of penicillin necessary to abort the infection, because of the *interim* multiplication of spirochetes *in vivo*. If there is a small inoculum and treatment during the incubation period, extraordinarily small doses of penicillin are sufficient to abort experimental infection of rabbits with syphilis. There is also suggestive evidence that these observations may be applicable to the problem of aborting human infections with syphilis.

Combining Chemotherapy With Penicillin, Arsenicals and Bismuth Compounds. In the pre-penicillin era, while use of one antisyphilitic agent alone was effective against syphilis, combined chemotherapy (arsenical and heavy metal) consistently seemed to give an ultimate advantage to the patient. Although penicillin alone in adequate dosages seems effective in early syphilis, the failure rates are approaching, if not exceeding those obtained prior to the advent of penicillin, albeit with lessened toxicity. In view of the demonstrated efficacy of combined therapy (arsenical and heavy metal) the penicillin investigating triumvirate of Eagle, Magnuson and Fleischman[16] set out to see whether there was a synergistic action of penicillin and mapharsen (oxophenarsine hydrochloride) in the treatment of experimental syphilis such as had been previously demonstrated by Eagle[15a] for metal chemotherapy. In this earlier study it was shown that moderate doses of bismuth and mapharsen used in conjunction are far more effective than even large doses of an arsenoxide used alone. In the present study Eagle and his colleagues identified a qualitatively similar and quantitatively even more pronounced additive effect when penicillin was supplemented by mapharsen. In the therapy of experimental rabbit syphilis small fractions of the curative doses of penicillin and of mapharsen were curative when used in conjunction. The two drugs together were so much more effective than either alone as to suggest more than a single additive effect was involved. Regardless of the implications of the already clearly demonstrated fact that, clinically, arsenical and penicillin used concurrently are an effective combination, the increased incidence of severe reactions is a limiting consideration of no small moment (Stokes, Beerman and Ingraham[52]).

The effectiveness of combining bismuth with penicillin in the treatment of syphilis has had little attention in the literature to this time. Kohmer and Rule,[26b] under the conditions of their study, showed that not only oxophenarsine hydrochloride by intravenous and intramuscular injection but bismuth and potassium tartrate in oil by intramuscular injection has decided synergistic or additive therapeutic effects in the treatment of acute syphilitic orchitis of rabbits with penicillin.

Fever. In their study on the effect of hyperpyrexia on the therapeutic efficacy of penicillin in experimental rabbit syphilis, Eagle, Magnuson and Fleischman[16a] set out to determine *in vivo* whether fever has a similar favorable effect in the therapeutic efficacy of penicillin which Eagle and Musselman[17b] had shown was the case *in vivo* and others had shown with the arsenicals.[39a] In general this study confirms *in vivo* the previously cited *in vitro* results that the rate at which the Reiter strain of cultured *Spir. pallida* are killed by penicillin, increases with temperature throughout the range of 8° to 40° C. (see above). When penicillin was injected intramuscularly in syphilitic rabbits every 2 hours for 16 injections, it required

30,000 and 60,000 u./kg. to cure 50% and 90% of the animals respectively. When the body temperature, during the administration of penicillin, was raised by approximately 3° to 4° F. over an average 10 hour period, the total curative dose fell to 8000 (CD_{50}) and 3000 (CD_{90}) u./kg. The 8- to 10-fold increase in the therapeutic efficacy of penicillin indicated by those figures is a minimum, since in the fever experiment, 75% of the total amount of the penicillin administered was concentrated in the 6 injections given over the 10 hour fever session. This asymmetric distribution would tend to diminish the overall efficacy of penicillin as such. The favorable effect of fever on the therapeutic activity of penicillin tends to reflect the enhanced spirocheticidal effect of penicillin at higher levels of temperature. In addition, fever may have a spirocheticidal action if its own, additive or synergistic.*

Penicillin Resistance. Using the criterion of treatment resistance which we have employed,[4] (failure of lesions to heal and persistence of organisms in lesions in spite of treatment), and not mere irreversibility of positive serologic reactions, it is evident that practically no clinical penicillin resistance has been observed. The only reported cases (there are certainly others, unreported) were by Moore[39b] and Tyson.[57] Moore's patient† had a cutaneous gumma which failed to respond to 4,800,000 units of penicillin but did heal promptly after several weeks of arsenical and bismuth therapy. Tyson's patient with early syphilis received 2,400,000 units of penicillin over a period of 5 days. The chancre did not heal, although the serologic reactions had become negative after several weeks. The reactions subsequently became positive and secondary lesions appeared. Combination of fever with an additional 600,000 units of penicillin gave an excellent response. In view of the uncertainties of the correct time-dosage relationship of penicillin, it is likely that while this represents clinical treatment resistance, the dosage (and quality of the drug used) may have been inadequate to control the progression rather than the development of an actual penicillin resistant strain of *Spir. pallida* induced by inadequate dosage or other factors presumed to induce this phenomenon.

In passing, but not entirely germane to the present topic, penicillin has been shown clinically to be effective in patients with manifestations of syphilis resistant to arsenical and heavy metal therapy (Noojin and co-workers,[42] and Nelson and co-workers[41]).

The first experimental evidence that spirochetes might develop penicillin-resistance was the study of Dunham, Hamre, McKee and Rake[12] who showed that inadequate therapy of syphilis in the rabbit resulted in the production of a more resistant strain of *T. pallidum.* This study emphasizes the importance of adequate initial therapy and raises the question of the effect of inadequate penicillin treatment for syphilis given to patients with gonorrhea who might also be in the incubation period of syphilis, on *T. pallidum.* Bessemans and Derom,[5c] in their second note, reported studies on the influence of subtherapeutic doses of penicillin administered during the incubation period of experimental rabbit syphilis. They observed that the incubation of syphilis in these subtherapeutic doses of penicillin did not abort the infection or render it asymptomatic (Eagle *et al.*[35]), but retarded the appearance of clinical manifestations. This again is no evidence in the direction of demonstrating a penicillin resistance, but perhaps is merely an expression of the spirochetistatic action of inadequate penicillin dosage. Tung and Frazier[56] did not note the development of increased resistance or tolerance to penicillin in cultures of the Reiter strain of *T. pallidum* after being subjected to 15 passages through media containing the preparation. Kolmer and Rule[26b] have produced experi-

* Craig, Schwemlein and Kendall: J. Lab. and Clin. Med., 30, 1016, 1945.
† Reported by Hahn: Am. J. Syph. Gonor. and Ven. Dis., 31, 542, 1947.

mental evidence against the existence of acquired penicillin resistance. They found that a minimal curative dose for acute testicular syphilis of rabbits (Nichols-Hough strain) of commercial penicillin in aqueous solution by intramuscular injection 3 times a day for 10 successive doses was apparently slightly more than 1000 u./kg. (10,000 units total) and probably a little less than 5000 units per dose (total dose: 50,000 units). This strain did not show any evidence of acquired resistance or tolerance to penicillin after 3 consecutive passages through the testicles of rabbits when they were treated with subcurative amounts of the compound. While the last word has not been said on the problem of penicillin resistant *Spir. pallida*, the lack of evidence from the clinical standpoint of real treatment resistance among so many patients with early syphilis undoubtedly treated unwittingly with an inadequate dosage, speaks eloquently against the possibility of such a phenomenon. The situation is well summarized by Tainter,[54] who stated that "there is as yet no absolute evidence that the development of penicillin resistant strains has occurred in clinical practice in any disease."

THE MODE OF ACTION OF PENICILLIN AGAINST T. PALLIDUM. Practically nothing of a definitive nature is known about the actual site of action or mechanism of action of penicillin on *T. pallidum*. The vast amount of experimental data already accumulated has seemed largely to define as precisely as possible the condition under which this new method of treatment works to the best advantage of eliminating infection with syphilis. In this connection Eagle, Magnuson and Fleischman[16c] have summarized our present knowledge so concisely that it is quoted *verbatim*.

"*Mode of Action of Penicillin.* The mode of action of penicillin differs from that of the arsenicals in several fundamental respects. When a trivalent arsenical such as mapharsen is injected, it is bound by the organisms in competition with the tissues. The combination is largely completed within a short time, and the spirochetes subsequently die or survive in relation to the amount so bound and their varying individual susceptibility. A single exposure to a temporarily high concentration of the arsenical apparently kills as many organisms as repeated exposures to small concentrations. It has been shown in both animals and man that the curative dose of mapharsen in syphilis is essentially the same whether the drug is administered in a single massive injection, in 10 to 20 large injections administered over a period of days, or in many small injections administered over a period of months. With penicillin, on the other hand, therapeutic efficacy was enormously enhanced merely by subdividing treatment into a large number of injections. The essential difference seems to be the fact that penicillin is not demonstrably bound and concentrated by the organisms, so that its action depends on the length of time for which effectively spirocheticidal levels are maintained in the body fluids. It is this introduction of the time factor, the necessity for maintaining an effective level, which primarily distinguishes the kinetics of the spirocheticidal action of penicillin from that of, *e. g.*, mapharsen.

"The therapeutic efficacy of penicillin, therefore, rests primarily on 2 factors: its concentration in the body fluids, and the period of time for which the organisms are exposed. Both factors contribute to therapeutic efficacy; but it is the clear implication of the experimental data here reported that of these 2 variables, the time factor is by far the more important."

A good summary of much of our present knowledge of the action of penicillin, especially on *T. pallidum*, has been prepared by Frazier and Frieden.[22] Since this compilation little has been added except to clarify certain minor points. Although the precise manner in which penicillin acts to destroy microörganisms is unknown, it is generally assumed that it interferes with the normal completion of some meta-

bolic transformation, thus inhibiting the development of the cell and leading to its eventual death. While it has been shown that penicillin undoubtedly has a direct spirocheticidal action *in vitro*, the reasoning with regard to the mechanism of this action follows somewhat along the lines proposed to explain that of the arsenicals. Because of this similarity, a brief statement of the mechanism of the antispirochetal action of the arsenical compounds is in order. It was thought for a long time that the arsphenamines did not have a direct action on spirochetes but became effective only when changed in the body to some active (avid form). This was postulated to be an arsenoxide. Eagle,[15b,c] however, initiated a critical series of *in vitro* studies which demonstrated that not only do the arsenicals have a direct antispirochetal action in concentrations effective only *in vitro*, but that tissue extractions (testicular) inhibited rather than enhanced this activity. Eagle then tested the hypothesis of Voegtlin, Dyer and Leonard[59] that arsenic may combine with the sulphydryl groups in the organism in the process of its destruction, and he found that various sulphydryl compounds almost completely abolished the spirocheticidal action of arsenicals and the heavy metals (mercury and bismuth). He believed that the rapid inactivation of the arsenical preparations by sulphydryl compounds indicated that the drugs owe their therapeutic activity to similar combinations with sulphydryl groups in the spirochetes. Penicillin too can be inactivated by various sulphydryl compounds. This has led Cavallito and Bailey[9] to believe that the main mode of action of many antibiotic substances rests in their facility to interfere with normal function of sulphydryl groups in bacterial metabolism. Chow and McKee[10] concluded that inactivation of penicillin by cysteine (a sulphydryl compound) may involve both the sulphydryl and the amino groups of the cysteine molecule. Leonard[29] suggested that "until it can be proven that penicillin binds to the SH groups of reduced bacterial protein in proportion to the number of SH groups in such protein . . . the actual point of attack of penicillin on microörganisms cannot definitely be concluded to be upon thiols of the organism." Cavallito,[8] on the other hand, disagrees with Leonard that penicillin needs to bind protein-SH in direct proportion to the number of SH groups in protein. Frazier and Frieden[22] summarize this problem as follows: "The similarity of the effects of sulphydryl compounds on penicillin and the arsenicals may be indicative of a possible similarity in the modes of action of the 2 types of antispirochetal agents. It is important to note, however, that significant differences exist with respect to such effects. While the inactivation of penicillin by cysteine proceeds slowly, the addition of this substance to suspensions of *T. pallidum* exposed to the action, for example, of arsphenamine, results in the immediate inhibition of the antispirochetal action. The fact that the arsenicals can be regarded as general protoplasmic poisons, while penicillin is not, may also be an indication of the essentially dissimilar nature of their respective modes of action."

The effects of penicillin on the morphology of various organisms have been reported repeatedly since the introduction of this type of chemotherapy.[7,11,19,20,23,38,60] Visible changes on the bacterial cell, inhibition of cell division, elongation and hypertrophy of the cell as well as changes in the actual shape of the bacterial cell have been observed by direct microscopic observation or by the study of electron microscopic pictures.

Since *T. pallidum* is a motile organism, direct darkfield and electron microscopic observation of the effects of penicillin on its morphology and motility promised to yield information of value in determining the sort and mode of antispirochetal activity. While a number of interesting observations have been made by Eagle, Dunham and Rake, and Frazier and his colleagues, their significance is not clear. Early in their work Eagle and his asso-

ciates, as previously noted, observed that *Spir. pallida* under the influence of doses of penicillin adequate to render the organisms non-viable remained actively motile for a period of 8 to 24 hours. Dunham and Rake observed in their *in vitro* studies that in cases where immobilization was incomplete, there was only sluggish motion. The last form of motion to be lost was bending of the body of the spirochete near the midpoint. They believed that it may well be that axial rotation is caused by the flagella revealed by the electron microscope, and that these are more sensitive to the action of impure penicillin than are the bodies of the organisms. The decrease in motility on exposure to partially purified penicillin suggested the possibility that these spirochetes might no longer be infectious. Even in the case where 90% of the spirochetes in the mixture used as inocula were immotile, there should have been more organisms than were necessary to cause infection if they had not been altered by exposure to the drug. As previously stated, spirochetes exposed to 1100 u./ml. of the partially purified preparation of penicillin are non-infectious for rabbits when injected intratesticularly, although many of them are still motile at the time of injection. Frazier and Frieden report that changes in the length of spirochetes were first observed in their laboratory by H. G. Sleeper. The relative distribution of long and short forms of the spirochete in material from early lesions observed in the darkfield, varied in such a way as to suggest that the changes observed were related to the effect of penicillin. Within 90 minutes after the first injection of 20,000 units of this drug, there was a relative increase in the number of the long forms of the spirochete. This increase was progressive until no more organisms could be found in the darkfield. This relative increase in long forms even occurred in a case when no such forms were seen before treatment. Frazier and Frieden[22] wonder whether this phenomenon of elongation of spirochetes may not be an indication of excessive growth and delayed cell division. Tung and Frazier[56] also made observations on penicillin sensitivity and morphology of the Reiter strain, of *T. pallidum* after cultivation in media containing penicillin. Electron micrographs of spirochetes grown in normal and penicillin-containing media were made with an RCA model electron microscope. They found that "While some of the spirochetes growing for the first time in media containing a sublethal concentration of penicillin became somewhat elongated, a tremendous increase in the length of individual organisms was observed in subcultures exposed to the same amount of penicillin, namely, from 0.05 to 0.1 u./ml. Under normal circumstances, the average length of the Reiter strain of spirochete is about 20 μ. Occasionally, organisms of 40 μ were seen in cultures exposed for the second time to penicillin, long forms of from 100 to 150 μ could easily be found in each oil immersion field. Most of the organisms were of the order of from 40 to 60 μ in length, but occasionally giant forms which had grown beyond the limits of the microscopic field were seen. . . . No statistical measurement was carried out, since no attempt was made to break up the large masses of tangled and matted forms composing the culture. The long forms had no appreciable increase in width. They appeared to be single organisms with stretched-out spirals and sluggish motility." The electron micrographs confirmed the impression that the long forms were single organisms. It is of interest to note that while the short spirochetes grown in normal media possessed flagella, none of the long forms were flagellated. Only an occasional, comparatively short spirochete in the midst of the long ones was found to have flagella.

"The life of the long forms were short. The elongated organisms appeared in the culture after 24 hours of incubation and gradually disappeared after 72 hours. A few moderately long spirochetes, measuring around 60 μ, were observed when the

organisms were transferred back to normal media after several passages through media containing penicillin.

"Although a few long forms were seen in cultures exposed for the first time to a sublethal concentration of penicillin, many more long forms began to appear after from 42 to 72 hours of incubation in cultures exposed to an inhibitory amount of the antibiotic agent (0.125 u./ml.). Most of the inoculated organisms survived a concentration of 0.125 u./ml. of penicillin and became elongated as the penicillin was vanishing from the culture medium. These long forms could be seen in the culture for about 4 days.

"In view of the possibility that commercial penicillin and crystalline penicillin might act differently on the spirochetes, as was shown with a pathogenic strain of *T. pallidum*, the experiment was repeated with penicillin sodium G having a potency of 1650 u./mg. It was found that both the pure and partially purified penicillin possessed the same potency of action and produced the same effect on cellular growth."

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OTO-RHINO-LARYNGOLOGY

UNDER THE CHARGE OF

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PATIENT'S PLIGHT, DOCTOR'S DESPAIR—THE COMMON COLD

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Often ignored because of the widespread belief that nothing much can be done about the common cold ("there is only one way to treat it—with contempt" is a practice as current today as it was in ancient Rome), the most democratic of ailments afflicts the average American from 1 to 4 times a year and represents an annual collective economic loss of $1,000,000,000. While the common cold rarely is a direct cause of death, it precedes or incites such serious infections as bacterial pneumonias, subacute bacterial endocarditis, septicemia and meningitis, and frequently precipitates the symptoms of coronary occlusion, peptic ulcer and other diverse diseases.[9]

Although it has never furnished the theme for a Hollywood motion picture or a year's run in the Broadway theatre, the common cold masks a human interest document that is neither prosaic nor lacking in drama. Infections of the respiratory tract, for example, cause more loss of time from industry and from schools than all other diseases combined.[6] Indeed, industry has lost 20 times as much time by the common cold as by accidents.[11] As has been said of the weather, so has been said of the common cold: "Everybody talks about colds but nobody does anything about them." The facts of the matter are: more has been done about the weather than about colds.

There are 2 purposes to this review: (1) to discuss briefly common cold knowledge; and (2) to propose a plan for mobilizing against the patient's plight. There are things the medical and allied professions can do beside despair.

Common Cold Background. It is generally held that the common cold is caused by a filtrable virus which is responsible for most colds occurring in epidemic form. Small outbreaks of colds, however, are sometimes caused by microörganisms capable of invading the healthy mucous membrane in the absence of a concurrent virus infection. In this country there are 3 main peaks of incidence each year: one occurs in October and November, a second early in January, and the third in March and April. The degree of susceptibility and resistance varies from one individual to another and from year to year. A certain degree of immunity develops as a result of a cold but it is of short duration, not more than several weeks or months after an attack.

Local resistance appears to be largely dependent on an intact mucous membrane, upon mucociliary activity, and presumably upon lysozyme.[2a] Local resistance may be impaired by structural abnormalities, such as nasal polypi, hypertrophied adenoid tissue, or chronic sinusitis where pathogenic bacteria are harbored and normal drainage is impaired. Although the precise rôle remains unclear, there is evidence that chilling the surface of the body causes a reflex vasoconstriction and a fall of temperature of the mucous membrane favoring the invasion of viruses.[7] Observations by Paul and Freese[8] suggest that sudden chilling of the body may bring on an attack in a person who has had a recent infection or a recent contact, but

only if such a condition accompanied the chilling.

It is generally assumed by most students of the common cold problem that abnormal vasomotor disturbances in the nasopharynx not only cause local injury to the tissue and facilitate infection by the pathogenic agents which are present, but that similar reactions may be caused by various irritants or by allergens in the air or food, or by injection. Furthermore, the vascular tone of the upper respiratory tract may be disturbed by systemic derangements and physiologic imbalances in the vegetative nervous system. Hence, such factors as constitution, fatigue and emotional strain are of etiologic importance in the common cold. In the few studies available, the histologic changes of the nasal mucous membrane in the common cold are almost identical with those of influenza in animals and in man.

It is reasonable to conclude that the common cold is not a definite single disease, but comprises a group of different affections.

Common Cold Therapy. Although methods for the prevention of colds have been directed along various lines, it can be stated unequivocally that none of the procedures is effective.

From time to time claims are advanced advocating a special diet either for the prevention or the cure of colds. The dietary measures most frequently recommended for the prevention of colds include a high protein diet, a diet low in carbohydrates, and large quantities of citrus foods, presumably to establish "alkalinization." None of these theories is supported by carefully controlled studies. Deficiency states may predispose to certain infections, but the supplying of extra vitamins and other food elements to the individual who shows no signs of deficiency disease does not protect him against colds. So widespread is the belief of the American public that the use of sodium bicarbonate or other alleged antacids is a preventive in the treatment of the common cold that it may really be called a part of American folklore. Diehl[1a] has demonstrated conclusively that the results obtained by "alkalinization" are of little or no value.

A number of procedures have been recommended periodically which have as their objective the conditioning of the body to sudden changes in external temperature of the skin, such as cold shower baths every morning and exercise outdoors followed by hot and cold shower baths. Controlled experiments have been carried out by Gafafer[3] who was unable to show any lower incidence of colds in people who followed these practices when compared with a group who did not.

In attempting to reduce the number of colds in susceptible persons, vaccines containing a wide variety of microörganisms commonly found in the respiratory tract have been employed. Extensive studies by Diehl and others[16] have demonstrated that cold vaccines, whether given parenterally or by nasal spray, do not prevent colds. As for cold vaccines given orally, the *Journal of the American Medical Association* has this to say editorially:[5] "Recent communications to the offices of the American Medical Association indicate that the prescription and sale of cold vaccines is again taking place on a large scale. This, in the face of the recognized lack of scientific evidence for the value of these preparations, is indication of irresponsibility on the part of some manufacturers of pharmaceuticals. The scientific evidence against the value of oral cold vaccines is overwhelming; consequently individual physicians and firms who deal in pharmaceuticals and who lend themselves to the wholesale uncontrolled distribution of such preparations are perpetrating an unwarranted commercial assault on the public pocketbook."

The employment of ultraviolet light or germicidal aerosols for sterilizing the air of rooms where crowding is prevalent is believed by some to offer a means for reducing the incidence of colds. Recent United States Public Health Service studies,[10] however, cast serious doubt on the hope that colds and other respiratory ail-

ments can be banished by the chemical treatment of air with glycol aerosols and irradiation with ultraviolet light.

The treatment of the common cold consists mainly in the relief of symptoms as they arise. Treatment should never be standardized but should be suited to the needs of each patient. During the earliest stages of the common cold the prime objective is to supply moisture by means of steam inhalations to the stricken upper respiratory passages. Alcohol has been utilized for generations to abort impending colds or to treat them. In reasonable doses it causes peripheral vasodilatation and reëstablishes circulation in chilled cutaneous and mucosal surfaces. Rest in bed, especially if fever is present, diminishes the severity of the common cold, limits its spread to others, and reduces the frequency of complications. It has stood the rigid test of time as a most sane and effective measure.[2b]

Little attention need be paid to fluids in the average uncomplicated cold. It is probably best to let thirst to a great extent determine the fluid intake. Cathartics, long employed as a home remedy for colds, have no value; their excessive use may lead to dehydration. Although salicylates and other fever-reducing medicaments have no effect on the infectious process, they do control headache and muscular aches. These should not be given routinely but only if necessary. Sulfonamide compounds and penicillin are ineffective in virus-initiated colds. Their employment in an uncomplicated case of the common cold may give rise to strains of drug-fast pathogenic bacteria resistant to treatment with either.

Even temporary relief from the discomfort of nasal obstruction justifies the use of proper nasal medication.[4] Nasal vasoconstrictors can satisfactorily relieve nasal congestion for varying periods of time and when judiciously employed often aid in promoting adequate drainage from the nasal sinuses. Liquid nasal medicaments which function on a rational, physiologic basis are compatible with ciliary activity, do not vary greatly in their pH (5.5 to 6.5) from that of normal nasal secretions, are isotonic, and are non-injurious and non-toxic. The continual use of nasal preparations containing mineral oil sometimes leads to the development of lipoid pneumonia.[2a]

THE NEED FOR MOBILIZED RESEARCH. Although organized science has succeeded in the solution of atomic energy, it has not solved the riddle of the common cold. The discovery of atomic energy was made possible because men who knew most about their specialties convened and pooled their knowledge. Whereas in the past fundamental discoveries have been made by isolated workers devoting their leisure time to research, present-day experience indicates that most progress comes from the coöperative effort of individuals having different training and background. Had individual specialists worked on the atomic energy problem piecemeal, it is a question whether the nation could have succeeded in its solution.

In the opinion of Fabricant,[2c] the common cold age-long mystery could be eradicated if the same sort of mobilized research that went into unlocking the atomic age were directed at the cause and cure of colds. If the experience of the past has any meaning, it signifies that colds cannot be eliminated by the efforts of single groups of scientists working in isolation. The common cold contains too many diverse factors for any one individual or group of individuals to solve. Much medical investigation of the common cold in the past has been characterized by inefficiency and *laissez-faire*. Most research men, if the truth be stated bluntly, have been wasting precious time, effort and money by pursuing their individualistic bents in isolated laboratories, clinics and hospitals. A perusal of the vast medical literature on the common cold speaks eloquently in support of such a contention.

The mere accumulation of facts is not enough. It is important to use them to establish new principles or to confirm old

ones. Scientists are well aware that not all discoveries are made to order and that some come off quite by accident. However, the chances of finding a means of curing colds are infinitely better under mobilized research than under the present system. Although lack of funds is one of the major factors in limiting mobilized research, a comprehensive plan by which the common cold may be attacked is just as essential as ample financial support. If the methods of several great industrial laboratories are adopted—and these have provided a number of fundamental discoveries—money invested in fostering unified research will be spent efficiently; there would be time for long-range study and money for apparatus and technical assistance. Instead of hit-or-miss investigation like much common cold research, there would be long-term concentration on the most promising clues.

Recent attempts to find a cure for the common cold have been proving difficult for the Common Cold Research Unit of the National Institute for Medical Research in England. While it has been claimed by spokesmen for this group that promising results are being obtained, a warning has been issued against any optimistic notion that a cure for colds could be discovered without much more research.

In the United States a sanely conceived program to investigate the common cold, according to Fabricant, should function either under Federal or private sponsorship or both. Since there is always the danger that the interests of research workers from a single specialty (epidemiologists, for example) may become narrow, a national convention of various interested investigators should be called to meet under one roof for the purpose of creating a unified research program. Those in attendance should come not only from the medical ranks, but from the allied worlds of chemistry, biology, pharmacology, physiology, bacteriology, pathology, engineering and architecture. Such a group could function democratically as a coördinated team with a unanimity that is now lacking. In convention, it would be necessary to emphasize principles, to point out to each other past errors of approach, and finally to agree on promising lines of investigation for dissemination to designated research centers throughout the country. Out of such a conference would perhaps come a well-knit program of research which conceivably could telescope decades of individualistic groping into years of coöperative endeavor. Funds for a mobilized research program could be obtained, once the need was widely proclaimed, from private individuals, public subscriptions, foundations and from the Federal government.

The responsibility for initiating a program of mobilized research on the common cold rests primarily with those forward-looking clinicians and basic medical scientists who realize the need for eradicating the common cold scourge.

The time has come for accepting responsibility.

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BOOK REVIEWS AND NOTICES

DIAGNOSIS AND TREATMENT OF MENSTRUAL DISORDERS AND STERILITY. By CHARLES MAZER and S. LEON ISRAEL. 2nd ed. Pp. 570; 133 ills. New York: Paul B. Hoeber, 1946. Price, $7.50.

THIS book was first published shortly before Pearl Harbor. That a 2nd edition should be needed so soon after the war is evidence that interest has remained keen in a field far removed from military medicine and surgery.

No small part of this interest has been due to the book itself. The authors' approach to the various problems of menstrual dysfunction is the eminently practical one of dealing with the primary symptoms that bring the patient to her physician. A concise and lucid exposition of the physiologic and pathologic factors underlying each symptom is followed by a rational program of treatment. Such a book has been badly needed in a field where there has been so much misunderstanding, and so much irrational usage of hormone therapy.

In the new edition full account has been taken of contributions that have been made to the subject during the war. These have been added not as mere addenda or new paragraphs but by incorporation in an extensively re-written text. The authors have therefore preserved the integration of material, and clarity of exposition that made the original edition so readable.

The book retains its pleasing format. It can be recommended highly to general practitioners and gynecologists alike.

C. B.

MONOCULAR VISION TRAINING. By MILDRED SMITH EVANS, Orthoptic Technician, Wilmer Institute of the Johns Hopkins Hospital. Pp. 93; 18 ills. Baltimore: Williams & Wilkins, 1946. Price, $3.00.

THIS book is essentially a work book for the use of children within a certain age group. In the opinion of the Reviewer the book would be most useful for children about 7, 8 or 9 years of age. It is for the development and improvement of vision in an amblyopic eye where the amblyopia is monocular in type and is associated with a strabismus. The book has been well planned, the type in the first part is large and boldfaced, that in the latter part becomes smaller. There is a series of 28 lessons so that exercises given in the instructions could be completed in approximately 1 month's time. The author has had long experience, and the book should be useful in its limited field.

W. F.

HENRY SEWALL, PHYSIOLOGIST AND PHYSICIAN. By GERALD B. WEBB and DESMOND POWELL. Pp. 191; 16 ills. London: Oxford Univ. Press. Baltimore: The Johns Hopkins Press, 1946. Price, $2.75.

THIS biography of a beloved American physician is a highly successful combination of sympathetic personal record and analysis of professional accomplishment. The authors have given an interesting account of Sewall's youth and education, his early and important, if too little known, studies on physiology and the fundamental aspects of immunology, and his later days as a distinguished authority on tuberculosis. In preparing the book they were in contact with many of Sewall's friends and scientific acquaintances, and as a result the book abounds in interesting personal reminiscences. Sewall was a scholar of first rank, a writer of genius, and an investigator of unusual perception, and always a gifted and delightful companion. His remarkable literary combination of colloquialism and scientific precision is illustrated by scores of pithy extracts from his papers. As the authors point out, his productivity and clarity of thinking were not impaired even in the final months of his long life. All who are interested in medical history should read the chapter on Sewall's investigations of the toxicity of snake venoms to obtain a proper perspective on the development of toxins and antitoxins. The book is to be commended particularly for its emphasis on the influence of Sewall's extraordinary personality in the stimulation of the younger scientific investigators of his day.

E. L.

NEW BOOKS

Cineplasty. By HENRY H. KESSLER, M.D., PH.D. Foreword by Ross T. MCINTIRE, Vice-Admiral (MC), USN. Pp. 201; 314 ills. Springfield, Ill.: Charles C Thomas, 1947. Price, $6.75.

Concise Anatomy. By LINDEN F. EDWARDS, PH.D., Professor of Anatomy, Ohio State Univ. Pp. 548; 324 ills. Phila.: Blakiston, 1947. Price, $5.50.

Histopathology of the Ear, Nose and Throat. By ANDREW A. EGGSTON, B.S., M.D., Director of Laboratories, Manhattan Eye, Ear and Throat Hospital, and DOROTHY WOLFF, A.B., M.A., PH.D., Research Investigator, Endaural Hospital, New York. Pp. 1080; 505 ills.; 28 color plates. Baltimore: Williams & Wilkins, 1947. Price, $18.00.

Cancer—Diagnosis, Treatment, and Prognosis. By LAUREN V. ACKERMAN, M.D., and JUAN A. DEL REGATO, M.D., Pathologist and Radiotherapist to the Ellis Fischel State Cancer Hospital. Pp. 1200; 749 ills., 42 in color. St. Louis: C. V. Mosby, 1947. Price, $20.00.

Osteotomy of the Long Bones. By HENRY MILCH, M.D., Fellow of the American Academy of Orthopedic Surgeons. Pp. 294; 181 ills. Springfield, Ill.: Charles C Thomas, 1947. Price, $6.75.

Signs and Symptoms, Their Clinical Interpretation. Edited by CYRIL MITCHELL MACBRYDE, A.B., M.D., F.A.C.P., Assistant Professor of Clinical Medicine, Washington University School of Medicine. Pp. 439; 74 ills.; 6 color plates. Philadelphia: J. B. Lippincott, 1947. Price, $12.00.

Forensic Medicine. By KEITH SIMPSON, M.D. (LOND.), Lecturer in Forensic Medicine to Guy's Hospital, London. Pp. 335; 114 ills. Baltimore and London: Williams & Wilkins, 1947. Price, $4.50.

Skin Manifestations of Internal Disorders (Dermadromes). By KURT WIENER, M.D., Dermatologist, Mount Sinai, Deaconess and Saint Michael's Hospitals, Milwaukee, Wis. Pp. 690; 400 ills.; 6 color plates. St. Louis: C. V. Mosby, 1947. Price, $12.50.

Petticoat Surgeon. By BERTHA VAN HOOSEN. Foreword by DR. A. E. HERTZLER. Pp. 324. Chicago: Pellegrini & Cudahy, 1947. Price, $3.75.

Characterization of Organic Compounds. By F. WILD, M.A., PH.D., F.R.I.C., Fellow and Tutor of Downing College, Cambridge. Pp. 306; 11 figs. Cambridge: The University Press. New York: The Macmillan Company, 1947. Price, $3.75.

A VALUABLE monograph for both research workers and students. After a discussion of the classification of organic compounds and the methods of separating mixtures, the various groups of organic compounds are presented in the 9 subsequent chapters. The author imparts a wealth of up-to-date well-documented information. The preparation of many modern reagents is described, yet old established methods are not neglected. This book also contains a concise discussion of the methods of determining physical constants such as melting point, boiling point, density, refractive index, optical rotation, etc. The work can be recommended unreservedly. M. E.

A Short Handbook of Practical Anesthetics. By HOEL PARRY-PRICE, M.R.C.S., L.R.C.P., D.A. (R.C.S.), Royal Berkshire Hospital. Foreword by CECIL P. G. WAKELEY, C.B., Consulting Surgeon to the Royal Navy. Pp. 127; 50 figs. Bristol, England: John Wright & Sons, Ltd., 1946. Price, $3.00.

THIS is not a text for students of anesthesia, but an interesting account of practical experience in 25 years of civilian and military anesthesia. Written in a lucid manner, the author's experiences are both interesting and comforting. Many practical points can be found in this volume that are useful in the art of anesthesia. R. D.

A Handbook for the Diagnosis of Cancer of the Uterus by the Use of Vaginal Smears. By OLIVE GATES, M.D., Pathologist, Massachusetts State Tumor Service; and SHIELDS WARREN, M.D., Assistant Professor of Pathology, Harvard Medical School. With a Foreword by GEORGE N. PAPANICOLAOU, M.D., PH.D. Pp. 182; 50 ills. Cambridge: Harvard University Press, 1947. Price, $4.00.

Introduction to Medical Psychology. By L. ERWIN WEXBERG, M.D., Director, Bureau of Mental Hygiene, District of Columbia. Pp. 171. New York: Grune & Stratton, 1947. Price, $3.50.

AN exceedingly clear, broad account of the psychology that is important for the medical student and practicing physicians. The author reports in an unbiased way the central ideas of Freud, Watson, Cannon, the Gestaltists and others, and creates an unexpected harmony between strange bed-fellows. Chapters of special interest describe the differing values in man's life, the apparatus of knowledge, and the prolonged childhood of man. E. B.

The Engrammes of Psychiatry. By J. M. NIELSEN, M.D., F.A.C.P., Associate Clinical Professor of Neurology and Psychiatry, University of Southern California; and GEORGE N. THOMPSON, M.D., formerly Chief of Psychiatric Service, Los Angeles County General Hospital. Pp. 509; 28 ills. Springfield, Ill.: Charles C Thomas, 1947. Price, $6.75.

Pharmakologie, Als Theoretische Grundlage Einer Rationellen Pharmakotherapie. By KNUD O. MØLLER, Professor der Pharmakologie an der Universität Kopenhagen. Pp. 744; 54 figs. Basel, Switzerland: Benno Schwabe & Co., 1947. (Imported by Grune & Stratton.) Price, Fr. 48.

NEW EDITIONS

Diseases of Children. Edited by DONALD PATERSON, M.D. (EDIN.), F.R.C.P., and ALAN MONCRIEFF, M.D. (LOND.), F.R.C.P. Vol. I. Contributions by 29 Contributors. 4th ed. Pp. 771; 154 ills. Baltimore and London: Williams & Wilkins, 1947. Price, $9.00.

Water Supply and Sewerage. By ERNEST W. STEEL, C.E., Consulting Engineer to Instituto Nacional de Obras Sanitarias, Venezuela; Member American Society of Civil Engineers. 2nd ed. Pp. 666; 264 figs. New York and London: McGraw-Hill, 1947. Price, $6.00.

The Development of Modern Medicine. By RICHARD HARRISON SHRYOCK. 2nd ed. (1st Borzoi ed.) Pp. 472; 10 ills. New York: Knopf, 1947. Price, $5.00.

May's Manual of the Diseases of the Eye. By CHARLES A. PERERA, M.D., Assistant Clinical Professor, College of Physicians and Surgeons, Columbia University. 19th ed. Pp. 521; 387 ills., 93 in color. Baltimore: Williams & Wilkins, 1947. Price, $4.00.

A Handbook of Ocular Therapeutics. By the late SANFORD R. GIFFORD, M.D., F.A.C.S., Professor of Ophthalmology, Northwestern University Medical School. Revised by DERRISK VAIL, M.D., D.O. (OXON.), F.A.C.S. 4th ed. Pp. 336; 66 ills. Philadelphia: Lea & Febiger, 1947. Price, $5.00.

Textbook of Medicine. By VARIOUS AUTHORS. Edited by SIR JOHN CONYBEARE, K.B.E., M.C., D.M. (OXON.); F.R.C.P., Physician to Guy's Hospital. 8th ed. Pp. 1170; 56 ills. Baltimore and London: Williams & Wilkins, 1946. Price, $8.00.

THIS textbook covers the entire subject of internal medicine and in addition includes chapters on neurology and mental diseases. Although in some instances Americans will disagree with the descriptions of clinical pictures and with recommendations for therapy, it may be regarded as a convenient reference for the British viewpoint on clinical medicine. W. S.

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